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ABOUT COVER

AIMS AND SCOPE

The primary aim of *World Journal of Gastrointestinal Oncology* (*WJGO*, *World J Gastrointest Oncol*) is to provide scholars and readers from various fields of gastrointestinal oncology with a platform to publish high-quality basic and clinical research articles and communicate their research findings online.

WJGO mainly publishes articles reporting research results and findings obtained in the field of gastrointestinal oncology and covering a wide range of topics including liver cell adenoma, gastric neoplasms, appendiceal neoplasms, biliary tract neoplasms, hepatocellular carcinoma, pancreatic carcinoma, cecal neoplasms, colonic neoplasms, colorectal neoplasms, duodenal neoplasms, esophageal neoplasms, gallbladder neoplasms, *etc*.

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ORIGINAL ARTICLE

Basic Study

Expression and significant roles of the long non-coding RNA CASC19/miR-491-5p/HMGA2 axis in the development of gastric cancer

Li-Xiang Zhang, Pan-Quan Luo, Zhi-Jian Wei, A-Man Xu, Tao Guo

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Abstract

BACKGROUND

Gastric cancer (GC) is a common malignant tumor, long non-coding RNA and microRNA (miRNA) are important regulators that affect tumor proliferation, metastasis and chemotherapy resistance, and thus participate in tumor progression. CASC19 is a new bio-marker which can promote tumor invasion and metastasis. However, the mechanism by which CASC19 affects the progression of GC through miRNA is not clear.

AIM

To explore the role of the CASC19/miR-491-5p/HMGA2 regulatory axis in GC.

METHODS

To explore the expression and prognosis of CASC19 in GC through clinical samples, and investigate the effects of inhibiting CASC19 on the proliferation, migration, invasion and other functions of GC cells through cell counting Kit-8 (CCK-8), ethynyldeoxyuridine, Wound healing assay, Transwell, Western blot and flow cytometry experiments. The effect of miR-491-5p and HMGA2 in GC

were also proved. The regulatory relationship between CASC19 and miR-491-5p, miR-491-5p and HMGA2 were validated through Dual-luciferase reporter gene assay and reverse transcription PCR. Then CCK-8, Transwell, Wound healing assay, flow cytometry and animal experiments verify the role of CASC19/miR-491-5p/HMGA2 regulatory axis.

RESULTS

The expression level of CASC19 is related to the T stage, N stage, and tumor size of patients. Knockdown of the expression of CASC19 can inhibit the ability of proliferation, migration, invasion and EMT conversion of GC cells, and knocking down the expression of CASC19 can promote the apoptosis of GC cells. Increasing the expression of miR-491-5p can inhibit the proliferation of GC cells, miR-491-5p mimics can inhibit EMT conversion, and promote the apoptosis of GC cells, while decreasing the expression of miR-491-5p can promote the proliferation and EMT conversion and inhibit the apoptosis of GC cells. The expression of HMGA2 in GC tissues is higher than that in adjacent tissues. At the same time, the expression level of HMGA2 is related to the N and T stages of the patients. Reducing the level of HMGA2 can promote cell apoptosis and inhibit the proliferation of GC cells. Cell experiments and animal experiments have proved that CASC19 can regulates the expression of HMGA2 through miR-491-5p, thereby affecting the biological functions of GC.

CONCLUSION

CASC19 regulates the expression of HMGA2 through miR-491-5p to affect the development of GC. This axis may serve as a potential biomarker and therapeutic target of GC.

Key Words: Gastric cancer; Long non-coding RNA CASC19; miR-491-5p; HMGA2; Prognosis

Core Tip: Gastric cancer (GC) is a common malignant tumor, long non-coding RNA and microRNA are important regulators that affect tumor proliferation, metastasis and chemotherapy resistance, and thus participate in tumor progression, this study aim to explore the role of CASC19/miR-491-5p/HMGA2 regulatory axis in GC, this article verify that CASC19 may promote the progression of GC by regulating the miR-491-5p/HMGA2 signal axis, which can be helpful for the diagnosis and treatment of GC.

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INTRODUCTION

Gastric cancer (GC) is the fifth most common malignant tumor in the world, almost 66% of cases occurred in developing countries[1,2]. GC has the characteristics of rapid progression, and easy recurrence after surgery, It has become one of the most important public health problems that endanger the health of people[3]. Due to the lack of specific symptoms of early GC, most patients often develop advanced disease, some patients even lost the opportunity to undergo surgical resection. Because molecular targeted therapy has important application value in gastrointestinal tumors[4], studying the molecular biological characteristics of GC cells is of great significance for finding new therapeutic targets and improving the diagnosis and treatment of GC. Long non-coding RNA (lncRNA) and microRNA (miRNA) are important regulators that affect tumor proliferation, metastasis, and chemotherapy resistance. LncRNA is a non-decoding single-stranded RNA with a length of more than 200 nucleotides[5]. *LncRNA* has always been considered as a gene transcription hindering substance. With the progress of research, the function of *lncRNA* has been continuously revealed. *LncRNA* can participate in epigenetic regulation of genes, transcription regulation, and participate in the process of diseases[6,7]. More and more evidences show that *lncRNA* can regulate tumor growth, metastasis, angiogenesis and other pathological processes in a variety of ways, and promote the occurrence and development of malignant tumors[8]. It is also used in tumor diagnosis, treatment and prognostic judgment[9,10]. The abnormal expression of *lncRNA* and its biological function is one of the hot spots in the molecular pathology of GC in recent years. CASC19 is a newly discovered *lncRNA* located in the 8q24 region. There are many related *lncRNAs* in this region that can promote tumor invasion and metastasis[11,12]. Studies have found that CASC19 is involved in colorectal cancer, lung cancer[13,14], *etc.* It is over-expressed in a variety of tumors, so the inhibition of CASC19 may become a potential target for tumor therapy. However, there is few study about the mechanism of CASC19 such as apoptosis and epithelial-mesenchymal transition (EMT) of GC cells[15]. Therefore, it is of great significance to study the role and mechanism of CASC19 in GC cells. Bioinformatics technology is an important means to predict and identify miRNA-*lncRNA* interactions. *LncRNA-CASC19* was input into the miRNA target prediction database, and miR-491-5p was listed among them, inferring the binding relationship exist. *MiRNAs* (small non-coding

RNAs) involved in various functions of apoptosis, proliferation and autophagy regulation[16,17]. *MiR-491-5p* belongs to the miR-491 family and is located at 9p21.3. It has been found to be down-regulated in various malignant tumors, including prostate cancer and osteosarcoma[18,19]. *MiR-491-5p* may regulate cell apoptosis, cell differentiation, invasion and radiosensitivity by silencing its target genes, and act as a tumor suppressor gene. At the same time, we also predicted the binding site between *miR-491-5p* and HMGA2 through software. The hyperkinetic protein family (HMG protein family) is divided into three sub-families: HMGA, HMGB and HMGN. Each family has different functions due to different structures. HMGA is composed of 4 molecules, HMGA1a, HMGA1b, HMGA1c and HMGA2. The human HMGA2 gene is located at chromosome band 12q14-15. HMGA2 is involved in various diseases. More and more studies have shown that HMGA2 can be used as an oncogene/tumor suppressor in various human cancers[20-22]. For example, elevated HMGA2 can lead to GC patients with metastasis and poor prognosis[23], and HMGA2 is associated with tumor EMT transformation and BCL2[24], HMGA2 can be used as a non-coding RNA target gene, it can play an important regulatory role in the occurrence and development of tumors[25]. Based on the above, we speculate that CASC19 may promote the progression of GC by regulating the *miR-491-5p*/HMGA2 signal axis, and affect the invasion and metastasis of GC.

MATERIALS AND METHODS

RNA sequencing analysis

Screening of *miRNA* download CASC19 targeting *miRNA* from ENCORI database (http://starbase.sysu.edu.cn/), screening of target genes and differential genes download the target gene of *microRNA491-5p* from the ENCORI database (http://starbase.sysu.edu.cn/). At the same time, The Cancer Genome Atlas database TCGA database (TCGA, http://cancergenome.nih.gov/) is used to obtain clinical information and transcriptome data of GC patients. There are 32 normal samples and 375 tumor samples. Gene Set Enrichment Analysis (GSEA) analysis was performed using gsea3 software (downloaded from Broad Institute), and $P < 0.05$ was statistically significant. tumor-infiltrating immune cell (TIC) content CIBERSORT calculation method to obtain the content of tumor infiltrating immune cells.

Patients and specimens

GC and corresponding normal tissues obtained from 72 patients undergoing radical GC surgical from the First Affiliated Hospital of Anhui Medical University between August 1, 2018 and December 1, 2018. Besides, 44 patients undergo surgery between October 2019 and December 2019 were analyzed retrospectively in this study for IHC. All patients signed an informed consent form, and this study was approved by the Ethical Committee of our hospital.

Cell culture

Gastric cell lines and GES-1 were obtained from the Department of Immunology of Anhui Medical University. The cell lines were cultured in Rosewell Park memorial institute-1640 (RPMI-1640, Hyclone, United States), added with 10% fetal bovine serum (China), 100 U/mL penicillin, and 0.1 mg/mL streptavidin. All cells supplemented at 37 °C with 5% CO_2.

Cell transfection

Si-CASC19, *miR-491-5p* mimics, *miR-491-5p* inhibitor, HMGA2 siRNA (si-HMGA2), and negative control (NC) were purchased from Shanghai Sheng gong Biological Engineering, the transfection was processed with lip3000. Over-expression of CASC19 was achieve by plasmid (pcDNA) which included the full-length of *lncRNA CASC19* complementary DNA (cDNA), the transfection was processed with lip3000 and P3000. The sequences were listed in the Supplementary Table 1.

Quantitative reverse transcription PCR

Extract total RNA from GC or adjacent tissues according to the operating steps of AxyPrep Total RNA Miniprep Kit Product Instructions. Total RNA of cells was obtained using AxyPrep kit: (United States, Axygen). cDNA was synthesized using a PrimeScript RT reagent kit (TaKaRa, Japan). Quantitative reverse transcription PCR (qRT-PCR) was conducted on the ABI StepOne Plus PCR System (Biosystem, United States). GAPDH was used as an internal control for mRNA. U6 was used as an internal control for miRNA. The standard 2-ΔΔCt method was used for gene expression. All primers are displayed in the Supplementary Table 2.

CCK8

GC cells were seeded into a 96-well plate in 24 h after transfection, cell counting Kit-8 (CCK-8; TargetMol, Shanghai, China) was used to test the cell viability according the instructions[26], CCK-8 reagent was added and then calculate the cell survival rate.

Western blot assay

Cells was washed with phosphate-buffered saline (PBS) for once, and then put RIPA into each well, incubate in a shake bed at 4°C for 30 min. Separate the protein in each sample with 10% SDS polyacrylamide gel. After that, transfer the protein into PVDF film. Blocking with 1 × Blotto for 2 h, incubate with antibody at 4°C overnight. Washed with 1 × TBST for 5 min four times, incubate with the second antibody at room temperature for 1.5 h. Washed with 1 × TBST for 5 min four times, ECL developer solution was used for color development (United States, ThermocFisher scientific company).

Image J software was adopted to quantify the gray value of protein.

Immunohistochemistry

Four percent paraformaldehyde (PFA) fixed the clinical samples, and then embedded in paraffin. Immunohistochemical staining of the samples was performed using primary antibodies overnight and then incubated with secondary antibody. A light microscope was used to obtain the images.

Ethynyldeoxyuridine proliferation assay

Two microliters of ethynyldeoxyuridine (EdU) was added to the transfected cells and continued to culture for 4h. Follow the instructions of BeyoClick™ EdU-555 Cell Proliferation Detection Kit (Shanghai Beyotime Company) to conduct the cells, Fluorescence detection can be performed under a fluorescence microscope.

Transwell invasion assay

This was performed using Transwell chambers (Corning Corporation, United States) with matrigel (BD Biosciences, United States). Add 600 ul of serum-containing medium to the lower chamber, add 100 ul of cell suspension to the upper chamber, following incubation for 24 h at 37°C, the cells remaining on the upper membrane were removed and those on the lower surface of the membrane were fixed by paraformaldehyde. The cells were then stained and photographed.

Wound healing assay

After transfection, the monolayer was gently and slowly scratched across the center of the attached cells (0 h). After scratching, the wells were gently washed twice with PBS to remove the detached cells and residual serum. Subsequently, all the wells were refilled with fresh medium without serum and cells were incubated for additional 24 h. Cell migration was photographed using microscopy and image J software was used to calculate the scratch area under the field of view.

Cell apoptosis

Digest the cells with 1 mL of trypsin-free digestion solution without EDTA, then add 500 ul of binding buffer to each tube in order to fully suspend the cells by pipetting, 5 ul Annexin V-FITC and 5 ul Propidium Iodide was mixed well and complete the flow cytometry detection within 1 h.

Dual-luciferase reporter assay

PmirGLO-CASC19/HMGA2-wt/mut (Shanghai, China) was constructed by cloning wild type or mutant CASC19/HMGA2 into the pmirGLO vector (Promega, United States). PmirGLO-CASC19/HMGA2-wt/mut was cotransfected with *miR-1277-5p* or NC into 293 T cells. Dual-Luciferase Reporter Assay System perform Luciferase activity test.

Xenograft tumor assay

Five weeks old healthy nude mice were randomly divided into two groups and fed adaptively for 1 week (the *si-CASC19* group and the *si-NC* group), 48 h after cell infection, 0.1 mL of cell suspension (containing 2 × 10^6 cells) was subcutaneously inoculated into the right lower limb of nude mice to establish a nude mouse model of GC xenografts. Tumor volume and mouse weight were measured every 3 d. After 4 weeks, the mice were routinely sacrificed, and the tumor tissue was removed and weighed. The tumor was divided into two parts, one was stored at -80°C and the other was stored in 4% PFA. All animal experiments were approved by the Ethics Committee of Anhui Medical University.

Statistical analysis

All R packages for statistical analysis are on the "R v4.0.3" platform. Comparison of genes in tumor and normal tissue using the "beeswarm" package, using the wilcox.test test, multi-GSE using the "plyr" package, "ggplot2" package, "grid" package and "gridExtra" package, the immune cell content of Differences using the "ggplot2" package, "ggpubr" package and "ggExtra" package; using SPPS23.0 and graphpad7 for statistical analysis, continuous count data are expressed by means and standard deviations, and comparisons are made using *t*-test or Mann-whitney, frequency comparison A chi-square test or a fisher test was used to compare the means of ≥ 3 independent groups using the analysis of variance test, and the Bonferroni correction test was used to compare within-group comparisons. $P < 0.05$ was considered statistically significant.

RESULTS

LncRNA CASC19 promotes GC progression

The cancer tissues and adjacent tissues of 72 patients with GC were collected, it was found that the expression level of CASC19 in cancer tissues was higher than that in adjacent tissues by reverse transcription PCR (RT-PCR; Figure 1A). According to the median expression of CASC19 in cancer tissues of patients with GC was divided into two groups (high and low), and then the correlation between the expression of CASC19 and clinical information was analyzed (Table 1). The T stage, N stage, and tumor size were correlated with CASC19 ($P < 0.05$). CASC19 expression [HR with 95%CI: 2.478 (1.071-5.736)], TNM stage [HR with 95%CI: 4.122 (1.243-13.672)] and tumor size [HR with 95%CI: 2.969 (1.189-7.415)] were prognostic factors for GC patients (Table 2, $P < 0.05$). Besides the expression level of CASC19 in GC cell lines was higher

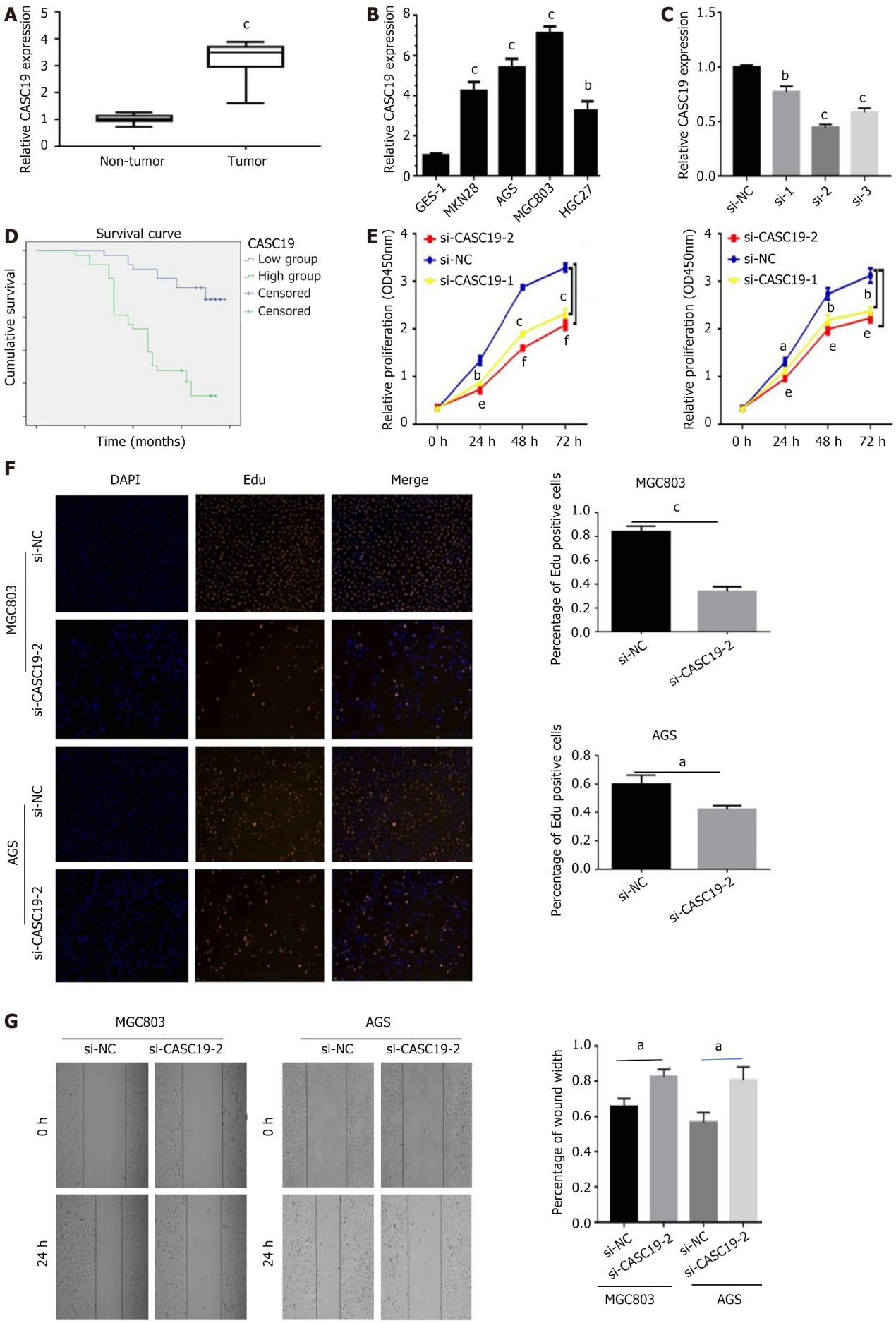
Table 1 Relationship between CASC19 and clinicopathologic characteristics

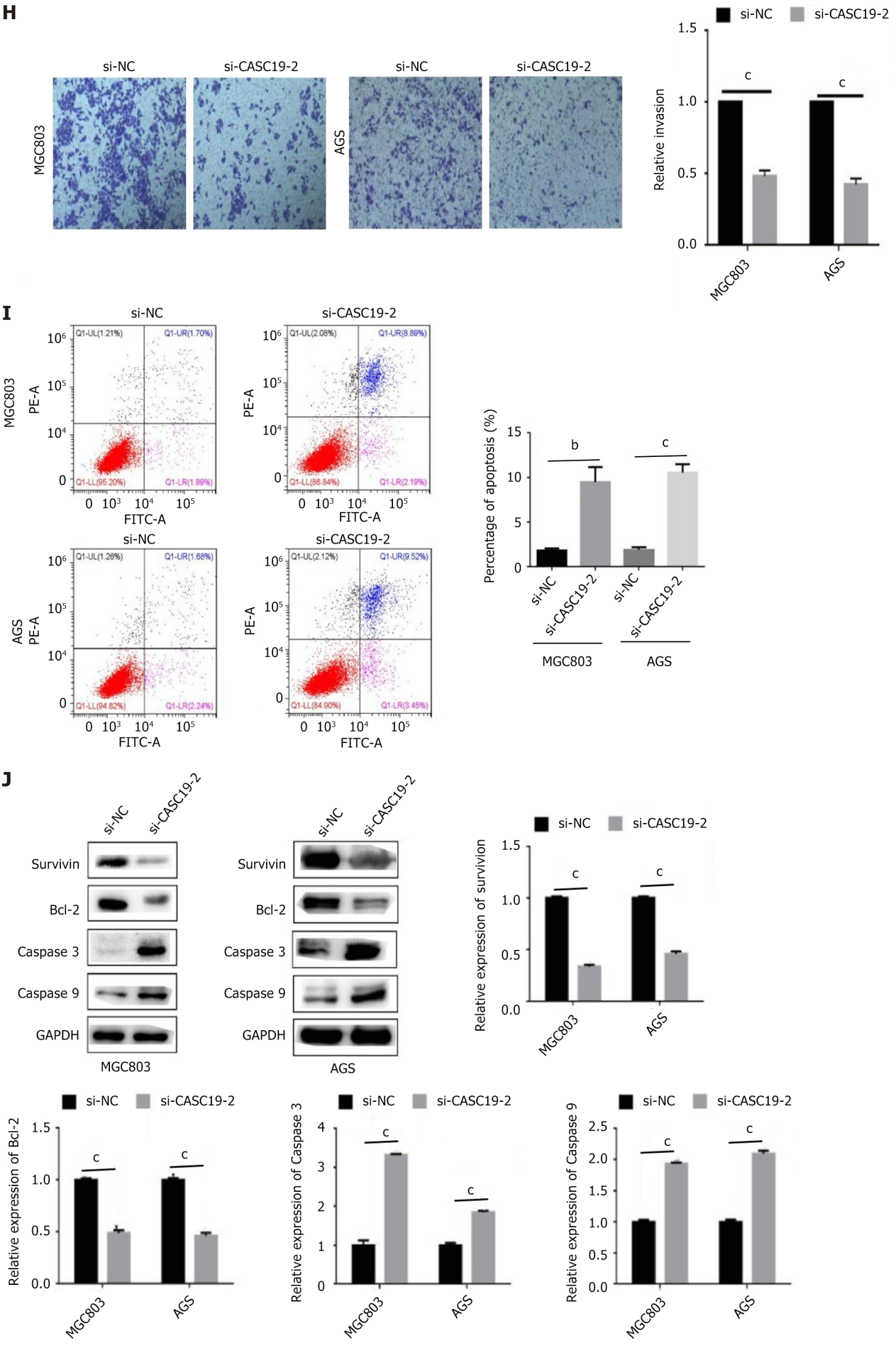
Patient-related factors	CASC19		χ^2	*P* value
	Low (*n* = 36)	High (*n* = 36)		
Gender			0.250	0.617
Male	25	23		
Female	11	13		
Age (years)			2.724	0.099
< 60	15	22		
≥ 60	21	14		
Tumor sizes			14.629	< 0.001
< 5 cm	29	13		
≥ 5 cm	7	23		
Differentiation grade			1.787	0.181
Poor	24	29		
Well	12	7		
Tumor location			5.077	0.079
Upper	3	3		
Middle	9	18		
Lower	24	15		
N stage			19.505	< 0.001
N0	22	4		
N1-N3	14	32		
T stage			9.000	0.003
T1-T2	18	6		
T3-T4	18	30		

Table 2 Risk factors for prognosis in patients with gastric cancer

Variable	Univariate analysis, HR (95%CI)	*P* value	Multivariate analysis, HR (95%CI)	*P* value
CASC19	5.986 (2.854-12.555)	< 0.001	2.478 (1.071-5.736)	0.034
Gender	1.056 (0.544-2.048)	0.872		
Age	1.407 (0.756-2.619)	0.282		
Differentiation grade	0.965 (0.471-1.976)	0.922		
Tumor size	9.438 (4.397-20.257)	< 0.001	2.969 (1.189-7.415)	0.02
Tumor location	0.736 (0.466-1.164)	0.19		
TNM (I, II/III)	10.293 (3.642-29.089)	< 0.001	4.122 (1.243-13.672)	0.021

than that in GES-1 ($P < 0.05$, Figure 1B). Lipofectamine 3000 was used to transfect *si-CASC19-1*, *si-CASC19-2*, *si-CASC19-3* into MGC803 cells and AGS cell lines, respectively, and the interference effect was verified by RT-PCR (Figure 1C). At the same time, the K-M survival curve was used to draw the survival curve of the high CASC19 group and low groups, as shown in Figure 1D, the prognosis of patients with low CASC19 expression was better. It was found that all three interfering siRNAs could reduce the expression of CASC19, of which *si-CASC19-2* had the best interference effect. Therefore, *si-CASC19-2* was selected to inhibit the expression of CASC19 in subsequent experiments. The effect of *si-CASC19-2* on cell proliferation was detected by CCK8 assay and EdU (Figure 1E and F), compared with the control group, the proliferation ability of MGC803 and AGS cells decreased after inhibiting CASC19. The Wound healing assay showed that reducing the expression of CASC19 could inhibit the migration ability of MGC803 and AGS cells (Figure 1G). The invasive ability of MGC803 and AGS cells decreased after inhibiting CASC19 by Transwell (Figure 1H). Flow cytometry


A
Relative CASC19 expression
Non-tumor
Tumor
B
Relative CASC19 expression
GES-1
MKN28
AGS
MGC803
HGC27
C
Relative CASC19 expression
si-NC
si-1
si-2
si-3
D
Survival curve
CASC19
Low group
High group
Censored
Censored
Cumulative survival
Time (months)
E
si-CASC19-2
si-NC
si-CASC19-1
Relative proliferation (OD450nm)
0 h
24 h
48 h
72 h
F
DAPI
Edu
Merge
MGC803
si-NC
si-CASC19-2
AGS
si-NC
si-CASC19-2
MGC803
Percentage of Edu positive cells
si-NC
si-CASC19-2
AGS
Percentage of Edu positive cells
si-NC
si-CASC19-2
G
MGC803
si-NC
si-CASC19-2
AGS
si-NC
si-CASC19-2
0 h
24 h
0 h
24 h
Percentage of wound width
si-NC
si-CASC19-2
si-NC
si-CASC19-2
MGC803
AGS


H
si-NC
si-CASC19-2
si-NC
si-CASC19-2
MGC803
AGS
si-NC
si-CASC19-2
Relative invasion
1.5
1.0
0.5
0.0
c
c
MGC803
AGS
I
si-NC
si-CASC19-2
MGC803
PE-A
FITC-A
Q1-UL(1.21%)
Q1-UR(1.70%)
Q1-LL(95.20%)
Q1-LR(1.89%)
Q1-UL(2.08%)
Q1-UR(8.89%)
Q1-LL(86.84%)
Q1-LR(2.19%)
AGS
Q1-UL(1.28%)
Q1-UR(1.68%)
Q1-LL(94.82%)
Q1-LR(2.24%)
Q1-UL(2.12%)
Q1-UR(9.52%)
Q1-LL(84.90%)
Q1-LR(3.45%)
Percentage of apoptosis (%)
15
10
5
0
b
c
si-NC
si-CASC19-2
si-NC
si-CASC19-2
MGC803
AGS
J
si-NC
si-CASC19-2
Survivin
Bcl-2
Caspase 3
Caspase 9
GAPDH
MGC803
AGS
Relative expression of survivion
Relative expression of Bcl-2
Relative expression of Caspase 3
Relative expression of Caspase 9
c
MGC803
AGS

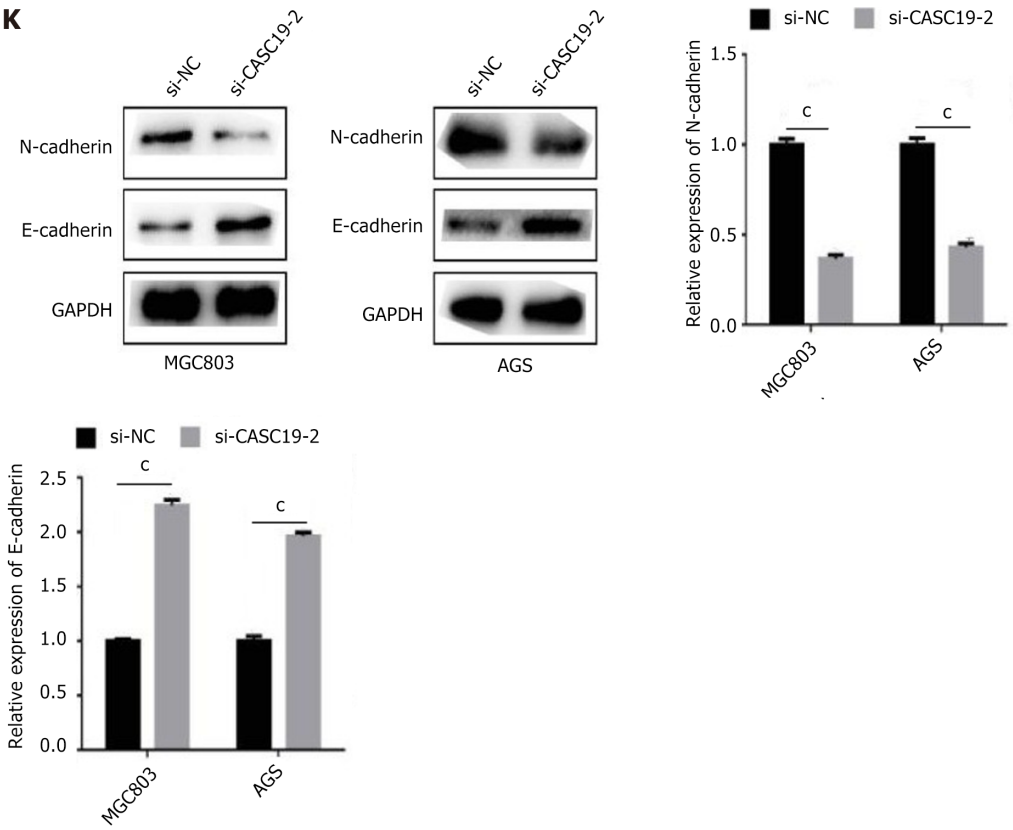
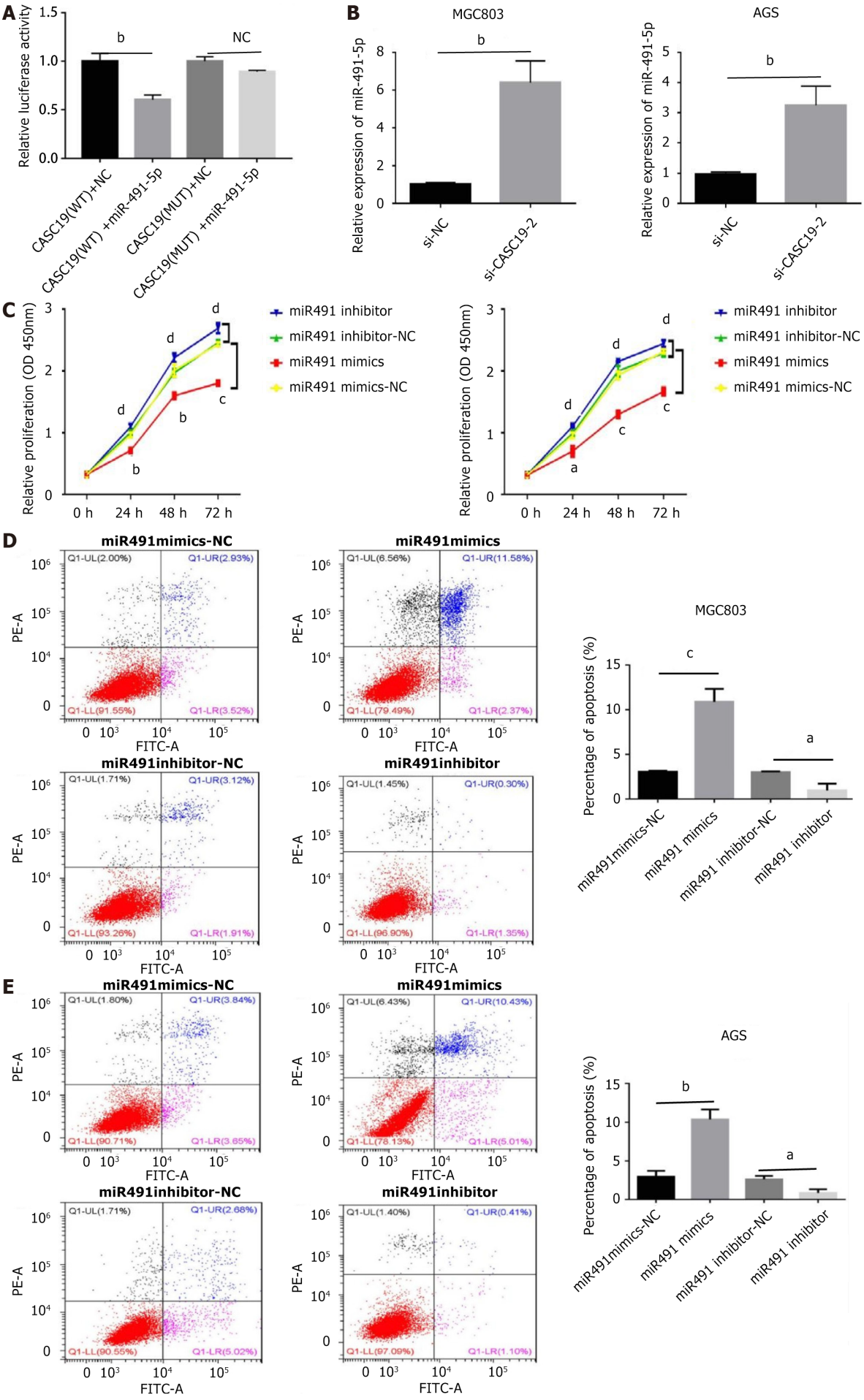


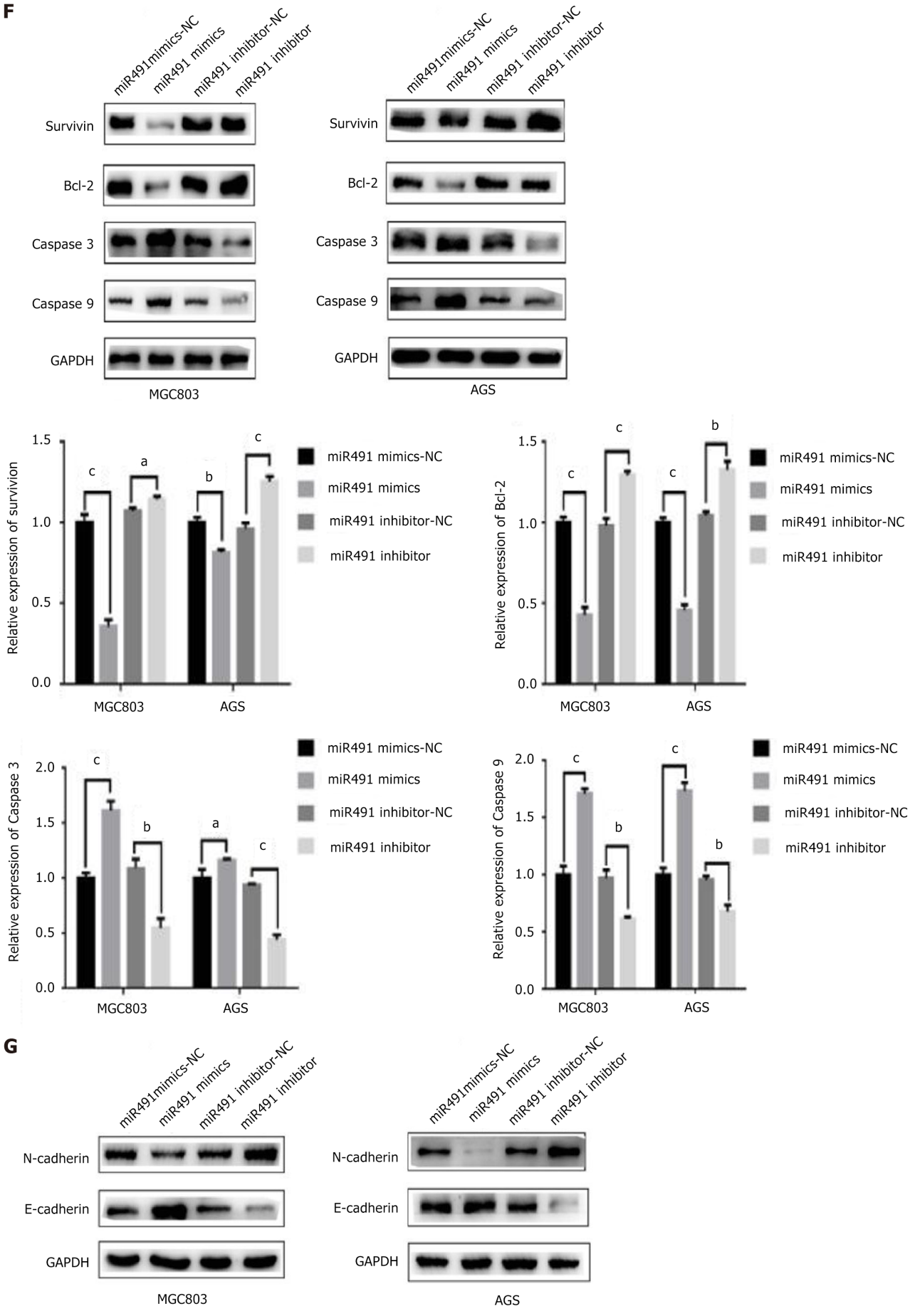
Figure 1 The clinical and biological function of CASC19 in gastric cancer. A: The expression of CASC19 in cancer tissues and adjacent tissues of 72 gastric cancer patients were measured by reverse transcription polymerase chain reaction; B: The expression level of CASC19 in gastric cancer cell lines and GES-1 was measured; C: The transfection result of si-CASC19-1, si-CASC19-2, si-CASC19-3 into MGC803 cells and AGS cell lines; D: The K-M survival curve of high CASC19 group and low groups; E: The effect of si-CASC19-2 on cell proliferation was detected by cell counting kit-8; F: The effect of si-CASC19-2 on cell proliferation was detected by ethynyldeoxyuridine; G: The result of wound healing assay after inhibiting CASC19; H: The invasive ability of MGC803 and AGS cells after inhibiting CASC19 by transwell; I: The cell apoptosis result after knockdown of CASC19 by flow cytometry; J: The level of apoptosis-related proteins were detected by Western blot experiment; K: The level of epithelial-mesenchymal transition-related proteins was examined by western blot. $^{a}P < 0.05$, $^{b}P < 0.01$, $^{c}P < 0.001$, $^{e}P < 0.01$, $^{f}P < 0.001$ was compared with the control group respectively. $P < 0.05$ was considered statistically significant.

was used to detect the results of cell apoptosis, knockdown of CASC19 could promote the apoptosis of MGC803 and AGS cells (Figure 1I). The level of apoptosis-related proteins were detected by WB experiment. After the expression of CASC19 was inhibited, the expressions of survivin and BCL-2 were decreased, and the expression of caspase3 and caspase9 proteins were raised (Figure 1J). The level of EMT-related proteins was also examined, compared with the control group, inhibiting the expression of CASC19 could reduce the expression of N-cadherin and increase the expression of E-cadherin (Figure 1K). These indicating that the potential of CASC19 was a prognostic biomarker in GC.

CASC19 sponges miR-491-5p and the effect of miR-491-5p in GC

ENCORI database showed that *miR-491-5p* may interact with CASC19, next, we verified the targeting relationship between CASC19 and *miR-491-5p* by dual luciferase reporter experiments. In 293T cells, co-transfected pmirGLO-CASC19-wt and *miR-491-5p* significantly reduced luciferase activity ($P < 0.05$), while co-transfection of pmirGLO-CASC19-mut and *miR-491-5p* had no significant effect on luciferase activity ($P > 0.05$, Figure 2A), suggesting that *miR-491-5p* is a direct targeting of CASC19. Knocking down the expression of CASC19 in MGC803 and AGS cells showed that the level of *miR-491-5p* was increased (Figure 2B), which further verified that CASC19 can negatively regulate the level of *miR-491-5p*. We verified the effect of *miR-491-5p* on the proliferation of GC cells by CCK8 assay. Figure 2C shows that enhancing the expression of *miR-491-5p* and inhibiting the expression of *miR-491-5p* can inhibit and promote the proliferation of GC cells, respectively. Flow cytometry showed that *miR-491-5p* mimics and *miR-491-5p* inhibitor can promote and inhibit GC cell apoptosis, respectively (Figure 2D and 2E). The level of apoptosis-related proteins was detected by WB experiment. The *miR-491-5p* mimics can decrease the expression of survivin and BCL-2, while caspase3 and caspase9 increased. Inhibiting the level of *miR-491-5p*, the expression of survivin and BCL-2 increased, while the expression of caspase3 and caspase9 decreased (Figure 2F). The level of EMT-related proteins was also detected by WB experiment. Compared with the control group, *miR-491-5p* mimics could reduce the expression of N-cadherin, increasing the expression of E-cadherin, *miR-491-5p* inhibitor can improve the level of N-cadherin, down-regulated the expression of E-cadherin (Figure 2G).


A
Relative luciferase activity
1.5
1.0
0.5
0.0
b
NC
CASC19(WT)+NC
CASC19(WT) +miR-491-5p
CASC19(MUT)+NC
CASC19(MUT) +miR-491-5p
B
MGC803
Relative expression of miR-491-5p
8
6
4
2
0
b
si-NC
si-CASC19-2
AGS
Relative expression of miR-491-5p
5
4
3
2
1
0
b
si-NC
si-CASC19-2
C
Relative proliferation (OD 450nm)
3
2
1
0
0 h
24 h
48 h
72 h
d
d
d
c
b
b
miR491 inhibitor
miR491 inhibitor-NC
miR491 mimics
miR491 mimics-NC
Relative proliferation (OD 450nm)
3
2
1
0
0 h
24 h
48 h
72 h
d
d
d
c
c
a
miR491 inhibitor
miR491 inhibitor-NC
miR491 mimics
miR491 mimics-NC
D
miR491mimics-NC
Q1-UL(2.00%)
Q1-UR(2.93%)
Q1-LL(91.55%)
Q1-LR(3.52%)
PE-A
FITC-A
miR491mimics
Q1-UL(6.56%)
Q1-UR(11.58%)
Q1-LL(79.49%)
Q1-LR(2.37%)
PE-A
FITC-A
miR491inhibitor-NC
Q1-UL(1.71%)
Q1-UR(3.12%)
Q1-LL(93.26%)
Q1-LR(1.91%)
PE-A
FITC-A
miR491inhibitor
Q1-UL(1.45%)
Q1-UR(0.30%)
Q1-LL(96.90%)
Q1-LR(1.35%)
PE-A
FITC-A
MGC803
Percentage of apoptosis (%)
15
10
5
0
c
a
miR491mimics-NC
miR491 mimics
miR491 inhibitor-NC
miR491 inhibitor
E
miR491mimics-NC
Q1-UL(1.80%)
Q1-UR(3.84%)
Q1-LL(90.71%)
Q1-LR(3.65%)
PE-A
FITC-A
miR491mimics
Q1-UL(6.43%)
Q1-UR(10.43%)
Q1-LL(78.13%)
Q1-LR(5.01%)
PE-A
FITC-A
miR491inhibitor-NC
Q1-UL(1.71%)
Q1-UR(2.68%)
Q1-LL(90.55%)
Q1-LR(5.02%)
PE-A
FITC-A
miR491inhibitor
Q1-UL(1.40%)
Q1-UR(0.41%)
Q1-LL(97.09%)
Q1-LR(1.10%)
PE-A
FITC-A
AGS
Percentage of apoptosis (%)
15
10
5
0
b
a
miR491mimics-NC
miR491 mimics
miR491 inhibitor-NC
miR491 inhibitor


F
miR491mimics-NC
miR491 mimics
miR491 inhibitor-NC
miR491 inhibitor
Survivin
Bcl-2
Caspase 3
Caspase 9
GAPDH
MGC803
AGS
Relative expression of survivion
Relative expression of Bcl-2
Relative expression of Caspase 3
Relative expression of Caspase 9
G
N-cadherin
E-cadherin

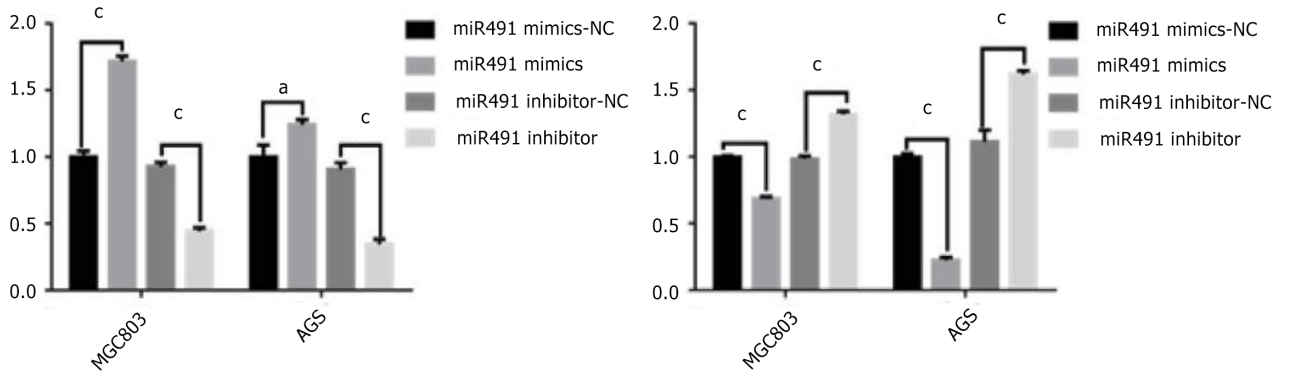


Figure 2 The effect of miR-491-5p in gastric cancer. A: The targeting relationship between CASC19 and miR-491-5p by dual luciferase reporter experiments; B: The expression of miR-491-5p after knock down of CASC19; C: The effect of miR-491-5p on the proliferation of gastric cancer cells by cell counting kit-8 assay; D and E: The consequence of apoptosis result by miR-491-5p mimics and miR-491-5p inhibitor; F: The change of apoptosis-related proteins by miR-491-5p mimics and miR-491-5p inhibitor through western blot experiment; G: The change of epithelial-mesenchymal transition proteins by miR-491-5p mimics and miR-491-5p inhibitor through western blot experiment. $^{a}P < 0.05$, $^{b}P < 0.01$, $^{c}P < 0.001$, $^{d}P < 0.05$ was compared with the control group respectively. $P < 0.05$ was considered statistically significant.

MiR-491-5p targets HMGA2 and the role of HMGA2 in GC

First, the targeting relationship between *miR-491-5p* and HMGA2 was verified by the dual luciferase reporter experiment. In 293T cells, co-transfected pmirGLO-HMGA2 3'UTR-wt and *miR- 491-5p* significantly reduced luciferase activity ($P < 0.05$), while co-transfection of pmirGLO-HMGA2 3'UTR-mut and *miR-491-5p* had no significant effect on luciferase activity ($P > 0.05$, Figure 3A), suggesting that HMGA2 is the downstream target gene of *miR491-5p*. In order to further study the regulatory relationship of *miR-491-5p* on HMGA2, MGC803 and AGS cells were transfected with *miR-491-5p* mimics and *miR-491-5p* inhibitor, respectively, and the level of HMGA2 was detected by RT-PCR (Figure 3B), *miR-491-5p* mimics could inhibit the expression of HMGA2, while *miR-491-5p* inhibitor could increase the expression of HMGA2, and this further verified *MiR-491-5p* targets HMGA2. The paraffin sections of cancer and adjacent cancer were obtained for immunohistochemical staining, and the results showed that the expression of HMGA2 in cancer tissue was higher than that in adjacent tissue (Figure 3C). All samples were scored by immunohistochemistry, according to the results of immunohistochemical scoring of cancer tissue patients, the patients were divided into high HMGA2 expression group and low expression group. The expression level of HMGA2 was correlated with the N stage and T stage of patients ($P < 0.05$; Table 3). The effect of HMGA2 on cell proliferation was detected by CCK8 assay, the proliferation ability of GC cells MGC803 and AGS decreased after inhibiting HMGA2 (Figure 3D). Flow cytometry was used to explore the regulation of HMGA2 about apoptosis, knockdown of HMGA2 can promote GC cell apoptosis (Figure 3E). The expression of HMGA2 in the GC tumor group and the normal group in the TCGA database was plotted by R language, and the difference was statistically significant (Figure 4A, $P < 0.05$), the relationship between HMGA2 and the clinical information of patients was also analyzed (Figure 4B), the expression of HMGA2 was correlated with the T stage of GC patients ($P < 0.05$), the gene enrichment analysis of HMGA2 (Figure 4C) was drew to predict the related pathways of HMGA2 such as chemokine signal (chemokine signal) and cell adhesion molecules. These pathways are associated with tumor progression [27]. The CIBERSORT calculation method was used to obtain the level of TICs, and the correlation between HMGA2 and immune cell content was analyzed (Figure 4D), the expression of HMGA2 was negatively correlated with B memory cells, mast cells and CD4 memory resting cells. The expression of HMGA2 was positively correlated with M1 macrophages, CD4 T memory activation cells and T follicular cells (Figure 4E), Therefore, the level of HMGA2 may be related to immune infiltration.

Vitro experiments proved the regulatory relationship between CASC19, miR-491-5p and HMGA2

Based on biological information, we predicted that CASC19 may regulate the level of HMGA2 through *miR-491-5p*. Among them, HMGA2 can regulate biological functions such as proliferation, invasion, migration, and apoptosis of GC, and it has been studied more in GC[28]. However, the function and mechanism of CASC19 in GC are not clear. Studies have shown that *miR-491-5p* regulates the target gene HMGA2 and thus affects tumor progression and invasion[29]. To demonstrate whether this effect depends on the regulation of *miR-491-5p*/HMGA2 pathway by CASC19, we used three cell lines. The expression of CASC19 was knocked down in MGC803 and AGS cells, and the expression of CAS19 was overexpressed in HGC27 cells, and then the relationship was verified by luciferase experiments, RT-PCR and Western blot experiments. Lipofectamine 3000 and p3000 were used to transfect pc-CASC19 (over-expression vector) into HGC27 cell lines, pc-CASC19 could significantly increase the expression of CASC19 (Figure 5A). MiR-491-5p containing HMGA2 3'UTR was co-transfected with pc-CASC19 or pc-NC in 293T cells (Figure 5B), the results showed that over-expression of CASC19 could block the effect of *miR-491-5p* on HMGA2 3'UTR. The relative inhibition of luciferase expression indicated that CASC19 could attenuate the effect of *miR-491-5p* on HMGA2 by competitively binding to *miR-491-5p*. MGC803 and AGS cells were transfected with *si-NC*, *si-CASC19-2*, co-transfected with *si-CASC19-2* and miR-491 inhibitor, respectively, The HGC27 cells were transfected with pc-NC, pc-CASC19, co-transfected with pc-CASC19 and *miR-491* mimics, respectively, and the expression of HMGA2 was detected by qRT-PCR (Figure 5C) and Western blot (Figure 5D).The

Table 3 Relationship between the HMGA2 and clinicopathologic characteristics

Patient-related factors	HMGA2		χ^2	*P* value
	Low (*n* = 22)	High (*n* = 22)		
Gender			0.109	0.741
Male	16	15		
Female	6	7		
Age (years)			1.467	0.226
< 60	10	14		
≥ 60	12	8		
Tumor sizes			0.910	0.340
< 5 cm	16	13		
≥ 5 cm	6	9		
Differentiation grade			3.536	0.060
Poor	11	17		
Well	11	5		
N stage			9.821	0.002
N0	13	3		
N1-N3	9	19		
T stage			4.956	0.026
T1-T2	11	4		
T3-T4	11	18		

above shows that CASC19 can competitively bind *miR-491-5p* to regulate the activity of HMGA2. In order to further verify CASC19 for the regulation of *miR-491-5p*/HMGA2, the following explores the ability of *miR-491-5p* to rescue CASC19 in GC cells. The cell proliferation ability was detected by CCK8, *si-CASC19-2* could inhibit the proliferation ability of MGC803 and AGS, and the addition of miR-491-5p could reverse the inhibitory effect of *si-CASC19-2*, for HGC27 cells with low expression of CASC19, the growth of HGC27 cells can be promoted by transfection of pc-CASC19, and the addition of *miR-491-5p* mimics can reverse the pro-proliferation ability of pc-CASC19 (Figure 6A). The migration ability of cells was detected by wound healing assay, *si-CASC19-2* could inhibit the migration ability of MGC803 and AGS, while adding *miR-491-5p* could reverse the inhibitory effect of *si-CASC19-2*, for HGC27 cells with low expression of CASC19, transfection of pc-CASC19 can promote the migration ability of HGC27 cells, and the addition of *miR-491-5p* mimics can reverse the migration-promoting ability of pc-CASC19 (Figure 6B). The invasion ability of cells was detected by transwell, and *si-CASC19-2* could inhibit the invasion and migration ability of MGC803 and AGS, while adding *miR-491-5p* could reverse the inhibitory effect of *si-CASC19-2*, for HGC27 cells with low expression of CASC19, transfection of pc-CASC19 can promote the invasive ability of HGC27 cells, while adding *miR-491-5p* mimics can reverse the invasive ability of pc-CASC19 (Figure 6C). Flow cytometry was used to detect cell apoptosis. Compared with the control group, knockdown of CASC19 could promote the apoptosis of MGC803 and AGS cells, while adding *miR-491* inhibitor could reverse the effect of si-CASC19-2 on cell apoptosis (Figure 6D). The level of apoptosis-related proteins were detected by Western blot experiment, after inhibiting the expression of CASC19, the expressions of survivin and BCL-2 were decreased, and the expression of caspase3 and caspase9 proteins were raised. After adding *miR-491-5p*, the expression levels of survivin and BCL-2 increased, while the expression levels of caspase3 and caspase9 decreased (Figure 6E). The level of EMT-related proteins was also detected by WB experiment, after inhibiting the expression of CASC19, the expression of E-cadherin was increased, and the expression of N-cadherin was decreased. After adding miR-491-5p, the expression of E-cadherin decreased, and the expression of N-cadherin increased (Figure 6F). Then HMGA2 can rescue the biological behavior of CASC19 in GC cells was also verified. AGS and HGC27 cells were transfected with pc-NC, pc-CASC19, co-transfected with pc-CASC19 and si-HMGA2, respectively. RT-PCR revealed that pc-CASC19 can promote the expression of HMGA2 compared with the control group, while adding si-HMGA2 can inhibit the effect of pc-CASC19 on HMGA2 (Figure 7A). Cell proliferation was detected by CCK8, pc-CASC19 could promote the proliferation of AGS and HCG27 cells, while adding si-HMGA2 can inhibit the effect of pc-CASC19 (Figure 7B). The migration ability of AGS and HGC27 cells was detected by wounding assay, the migration ability of AGS and HGC27 were promoted by pc-CASC19, whereas addition of si-HMGA2 reversed the pro-migratory capacity of pc-CASC19 (Figure 7C and D). The invasive ability of the cells was detected by transwell, and the transfection of pc-CASC19 could promote the invasive ability of cells, whereas addition of si-HMGA2 reversed the pro-invasive ability of pc-CASC19 (Figure 7E).

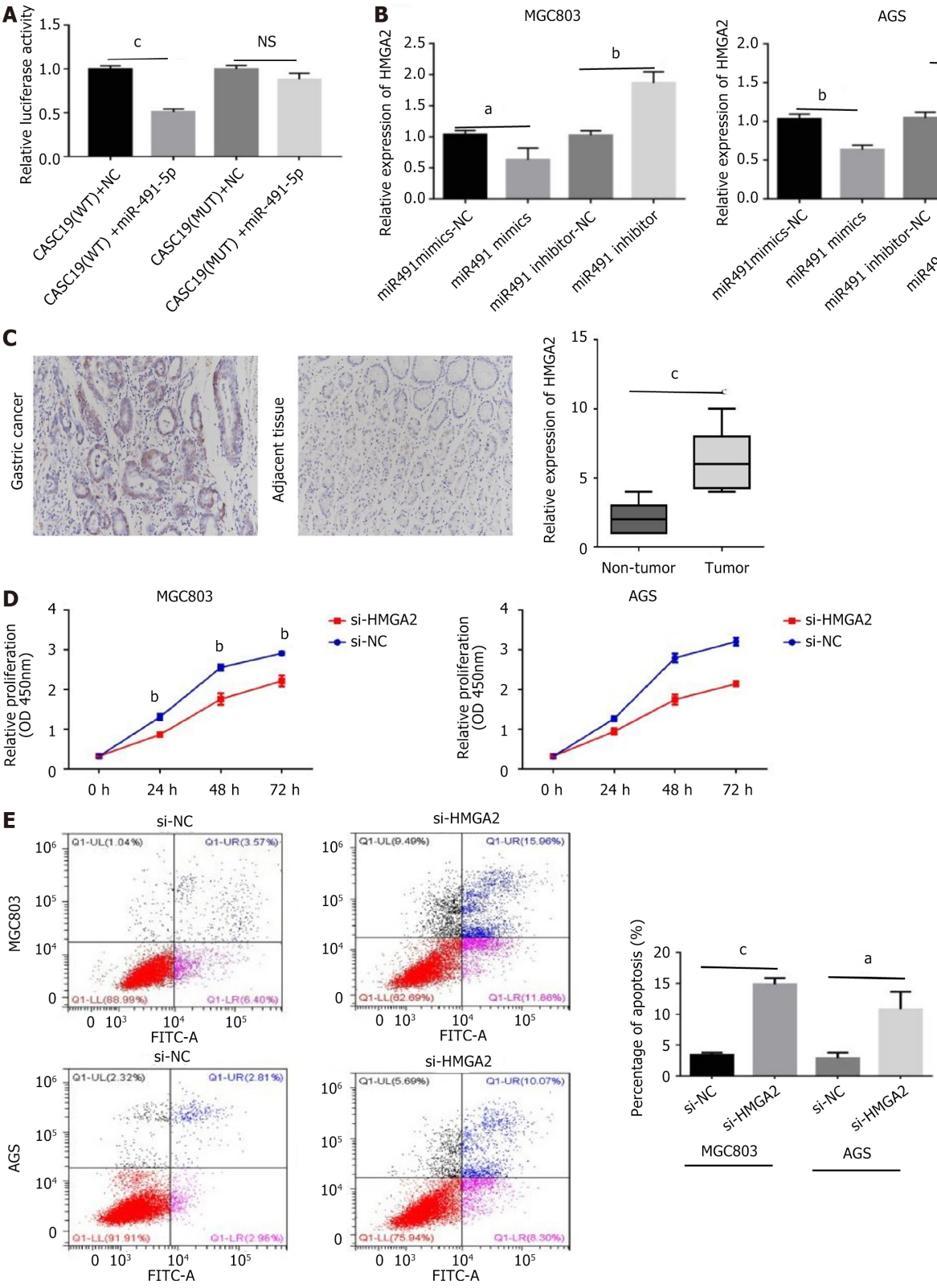
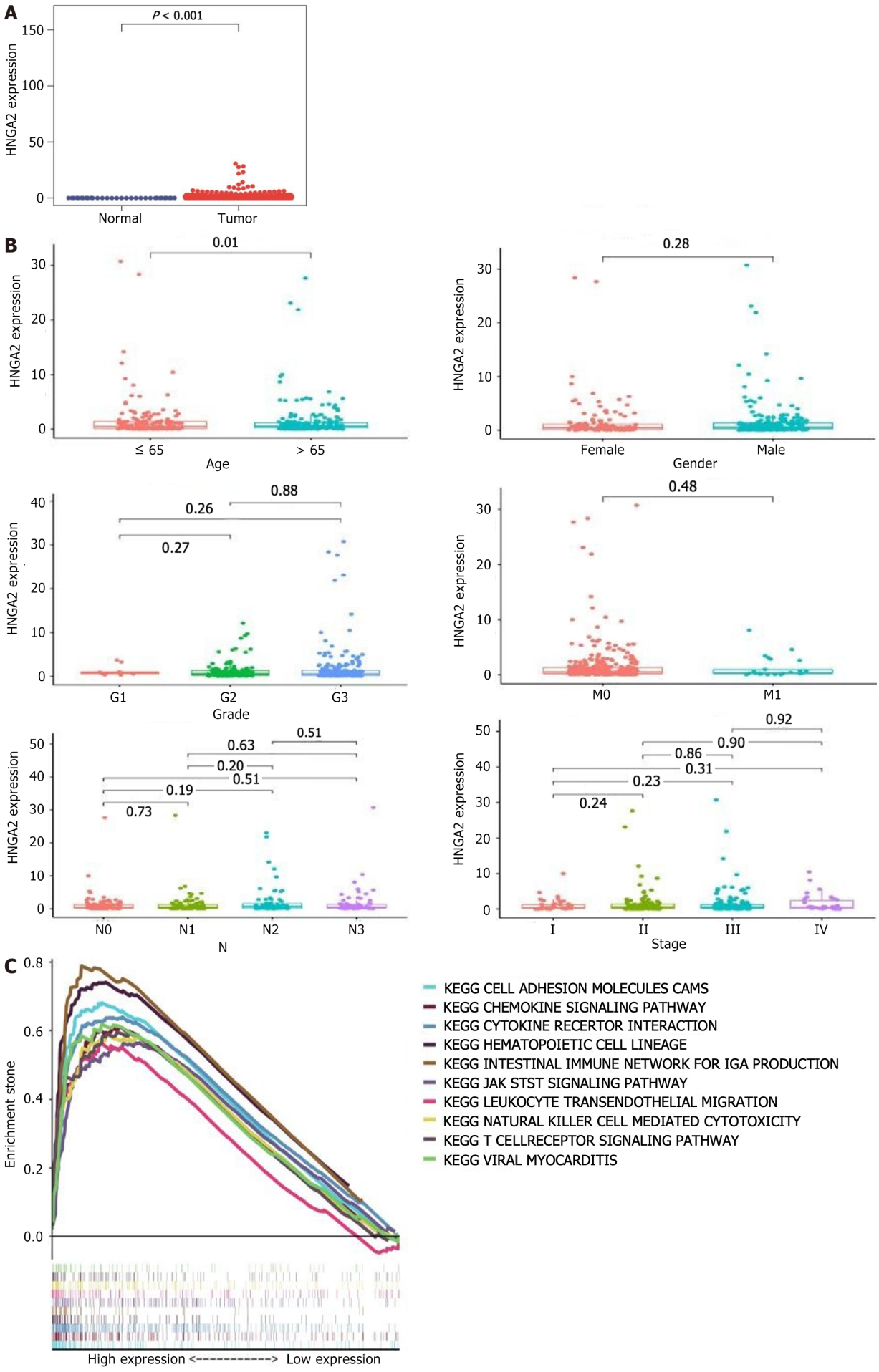


Figure 3 The role of HMGA2 in gastric cancer. A: The targeting relationship between miR-491-5p and HMGA2 by the dual luciferase reporter experiment; B: The expression of HMGA2 affected by miR-491-5p mimics and miR-491-5p inhibitor through reverse transcription polymerase chain reaction; C: The immunohistochemical staining of cancer and adjacent tissue; D: The effect of HMGA2 on cell proliferation was detected by cell counting kit-8 assay; E: Flow cytometry was used to explore the regulation of HMGA2 about apoptosis. $^{a}P < 0.05$, $^{b}P < 0.01$, $^{c}P < 0.001$ was compared with the control group respectively. $P < 0.05$ was considered statistically significant.


A
P < 0.001
HNGA2 expression
Normal
Tumor
B
0.01
HNGA2 expression
≤ 65
> 65
Age
0.28
Female
Male
Gender
0.88
0.26
0.27
G1
G2
G3
Grade
0.48
M0
M1
0.51
0.63
0.20
0.51
0.19
0.73
N0
N1
N2
N3
N
0.92
0.90
0.86
0.31
0.23
0.24
I
II
III
IV
Stage
C
Enrichment stone
High expression <----------> Low expression
KEGG CELL ADHESION MOLECULES CAMS
KEGG CHEMOKINE SIGNALING PATHWAY
KEGG CYTOKINE RECERTOR INTERACTION
KEGG HEMATOPOIETIC CELL LINEAGE
KEGG INTESTINAL IMMUNE NETWORK FOR IGA PRODUCTION
KEGG JAK STST SIGNALING PATHWAY
KEGG LEUKOCYTE TRANSENDOTHELIAL MIGRATION
KEGG NATURAL KILLER CELL MEDIATED CYTOTOXICITY
KEGG T CELLRECEPTOR SIGNALING PATHWAY
KEGG VIRAL MYOCARDITIS

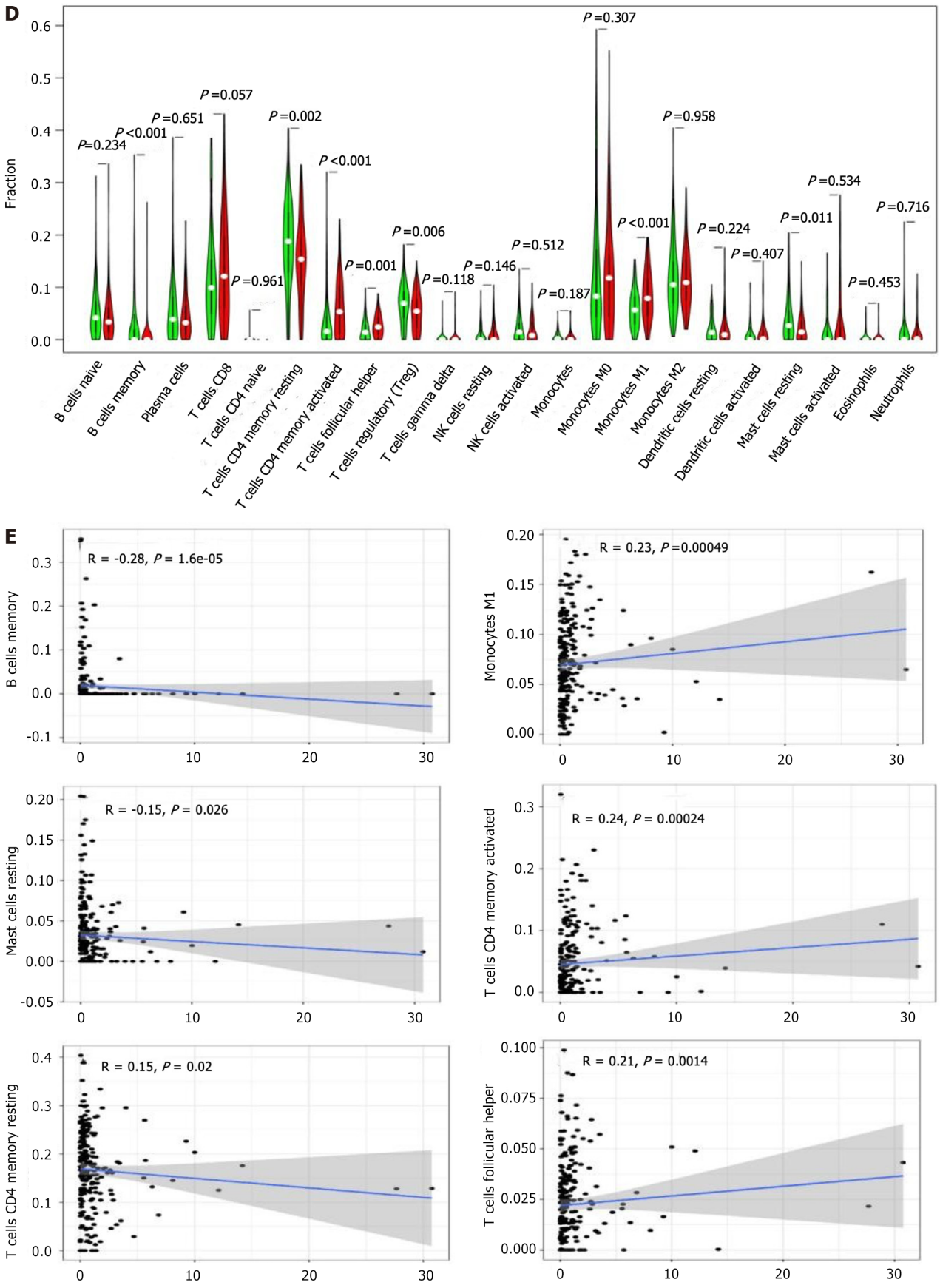


Figure 4 The value of HMGA2 in The Cancer Genome Atlas data. A: The expression of HMGA2 in the gastric cancer tumor group and the normal group in the TCGA database; B: The relationship between HMGA2 and the clinical information of patients; C: The gene enrichment analysis of HMGA2; D and E: The correlation between HMGA2 and immune cell level was analyzed.

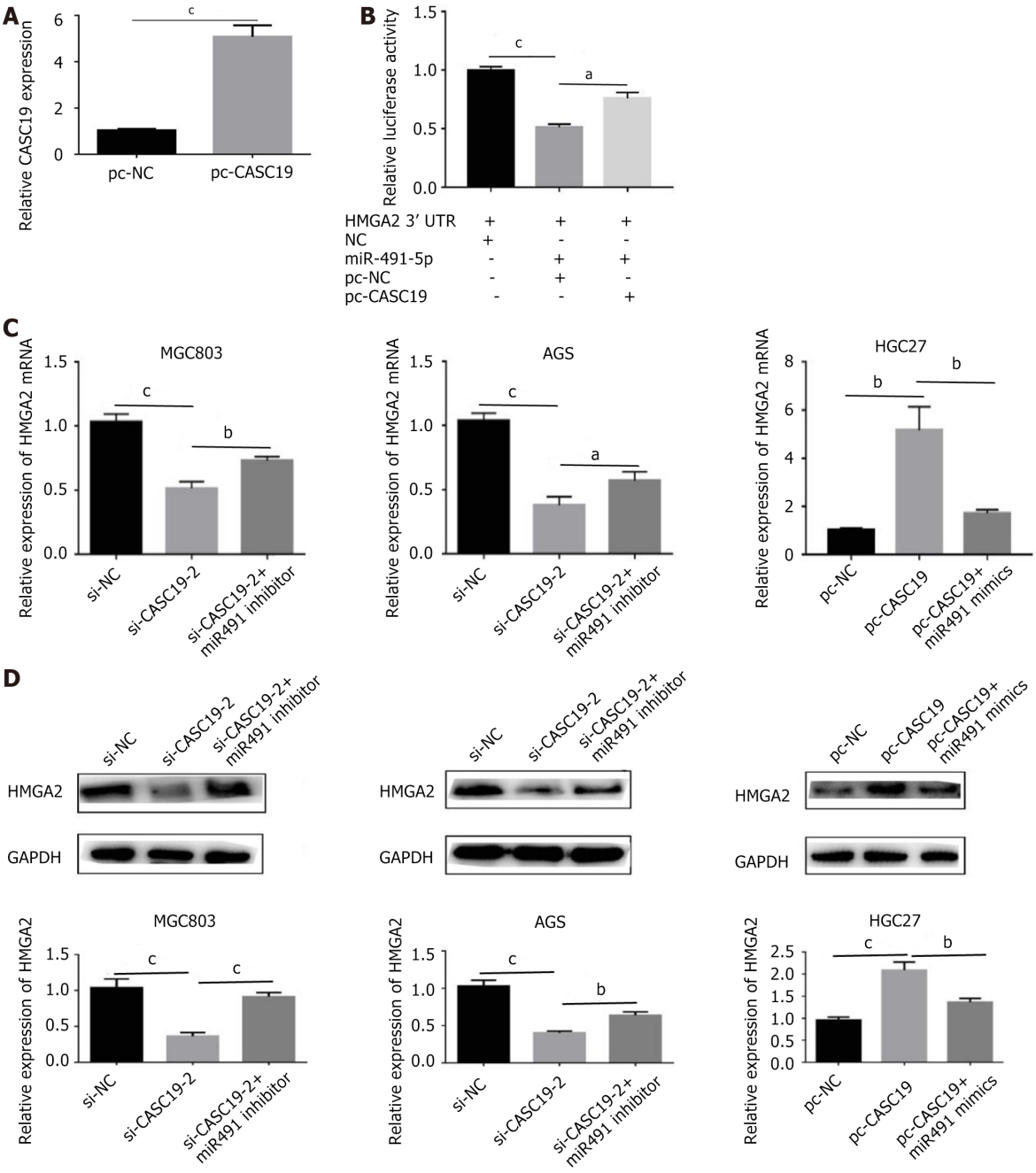
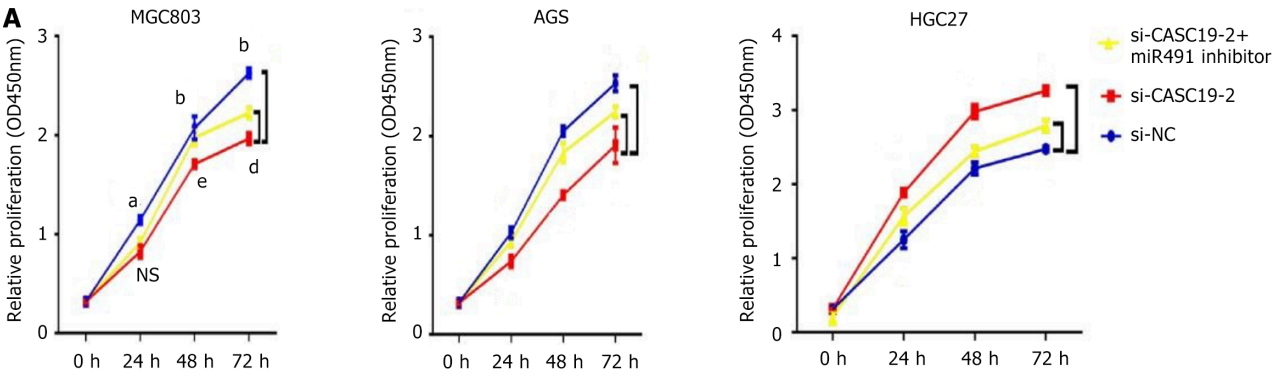
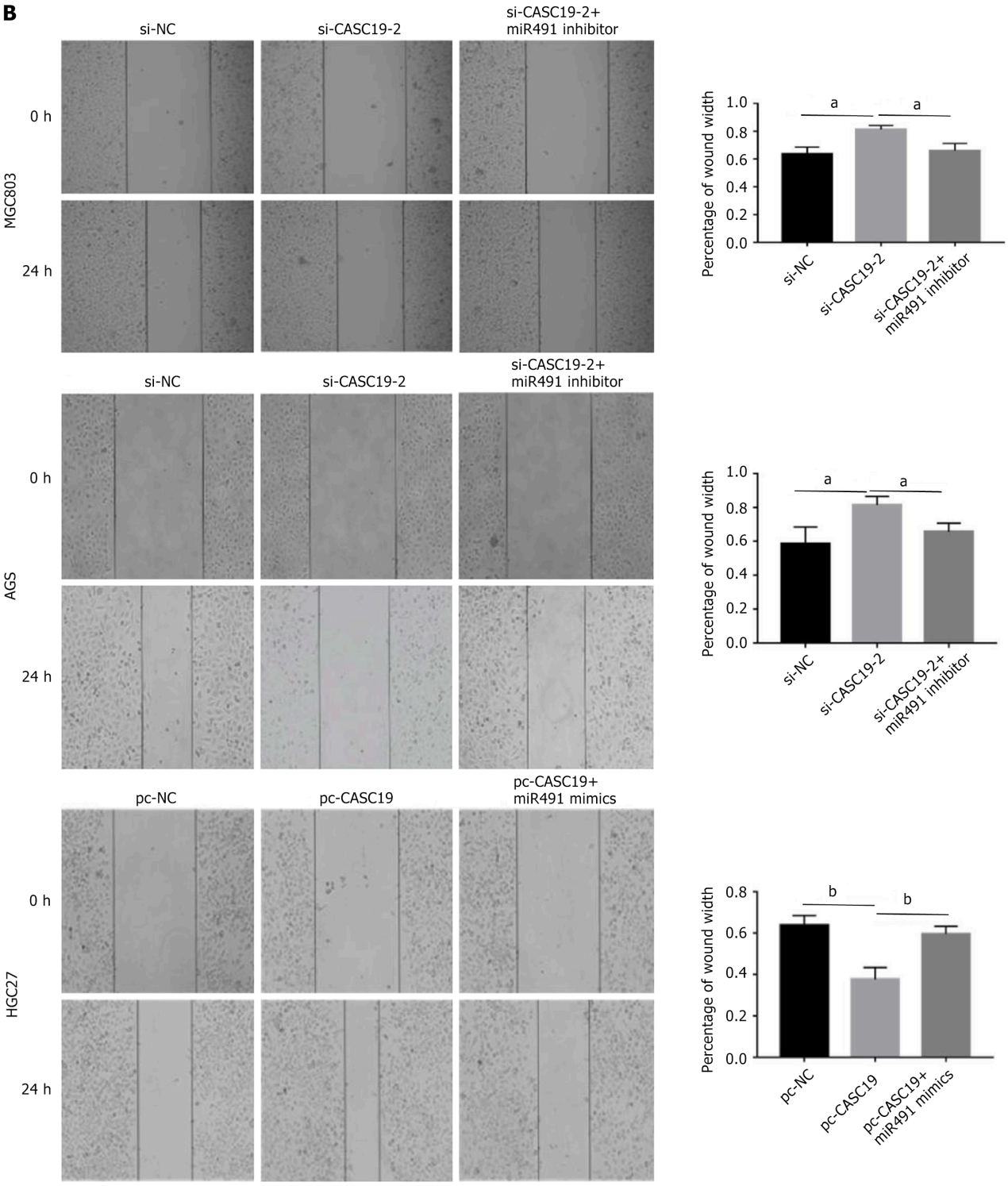


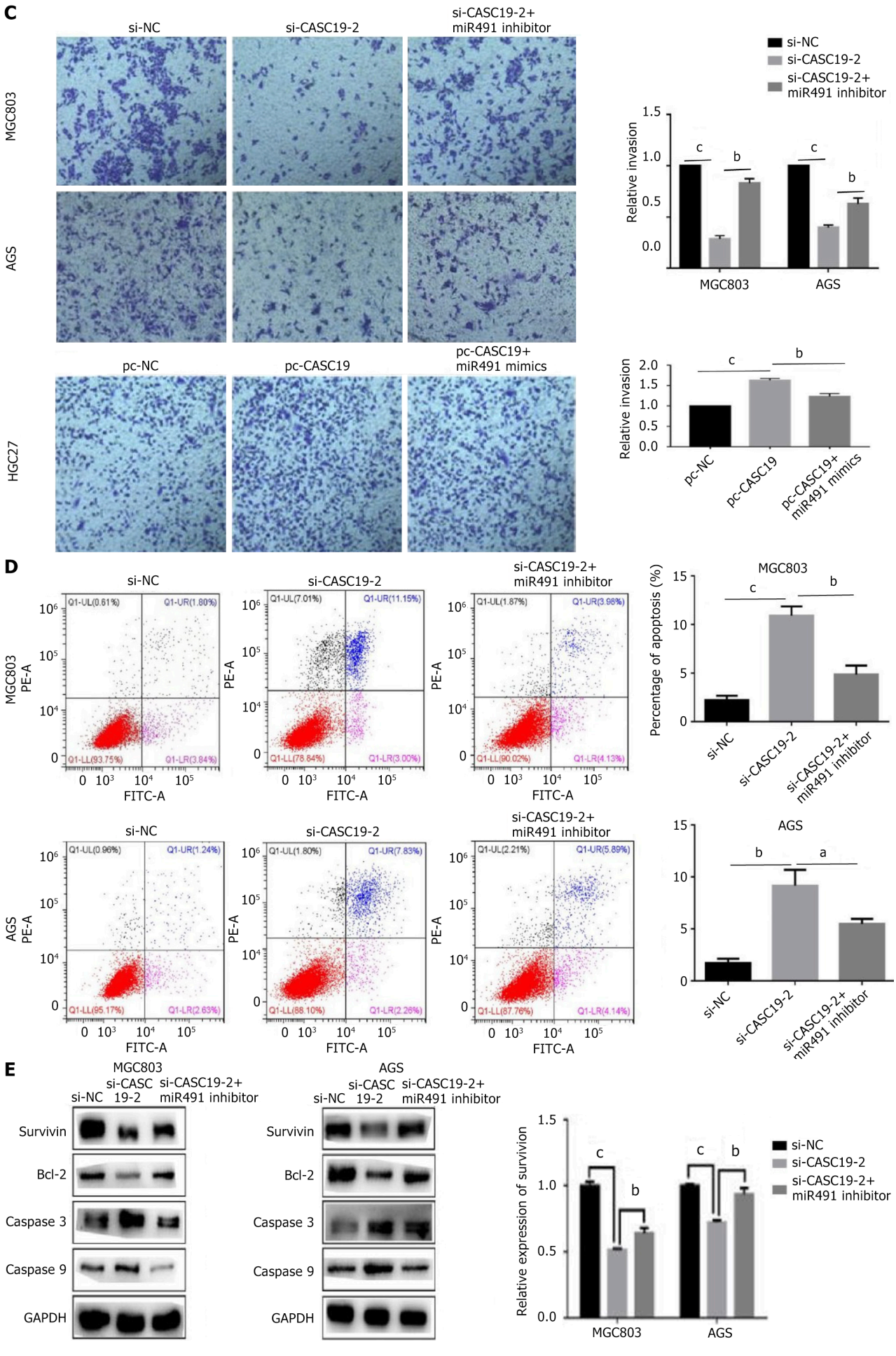
Figure 5 The regulatory relationship between CASC19, miR-491-5p and HMGA2 *in vitro*. A: The CASC19 expression of pc-CASC19 (over-expression vector) in HGC27 cell lines; B: The relative inhibition of luciferase expression of the miR-491-5p containing HMGA2 3'UTR co-transfected with pc-CASC19 or pc-NC in 293T cells; C and D: The expression of HMGA2 detected by quantitative reverse transcription polymerase chain reaction and western blot, MGC803 and AGS were transfected with si-NC, si-CASC19-2, co-transfected with si-CASC19-2 and miR-491 inhibitor, respectively, the HGC27 cells were transfected with pc-NC, pc-CASC19, co-transfected with pc-CASC19 and miR-491 mimics, respectively. $^{a}P < 0.05$, $^{b}P < 0.01$, $^{c}P < 0.001$ was compared with the control group respectively. $P < 0.05$ was considered statistically significant.

The oncogenic mechanism of CASC19 to GC in vivo

The efficiency of lentivirus silencing CASC19 was verified by RT-PCR. As shown in Figure 8A, after lentivirus transfected AGS cells, the expression of CASC19 in the cell line decreased significantly, indicating that the stable transfection was successfully constructed. In order to detect the effect of silencing CASC19 in animals, the AGS cells of the NC group and CASC19 silencing lentivirus infection group were inoculated subcutaneously in the right lower limb of nude mice, and the volume changes of the mice were observed every 3 d and there was little difference. The tumor volume was measured every 3 d. After 4 wk, the mice were routinely sacrificed. The tumor tissue was stripped and weighed, the tumor growth rate of the CASC19 silencing lentivirus infection group was slower, and the weight was significantly smaller than that of the NC group (Figure 8B). HE staining of the tumor showed that after down-regulating the expression of CASC19, the number of tumor tissue necrosis was more, and malignant morphology such as tumor volume could be suppressed (Figure 8C). Tumors were stained with TUNEL, the expression of CASC19 was down-regulated, and apoptotic cells in tumor tissues were significantly increased (Figure 8D). The tumor was subjected to immunohistochemical staining, and


A
MGC803
AGS
HGC27
Relative proliferation (OD450nm)
0 h
24 h
48 h
72 h
a
b
d
e
NS
si-CASC19-2+ miR491 inhibitor
si-CASC19-2
si-NC


B
si-NC
si-CASC19-2
si-CASC19-2+ miR491 inhibitor
MGC803
AGS
HGC27
0 h
24 h
pc-NC
pc-CASC19
pc-CASC19+ miR491 mimics
Percentage of wound width
a
b


C
si-NC
si-CASC19-2
si-CASC19-2+ miR491 inhibitor
MGC803
AGS
si-NC
si-CASC19-2
si-CASC19-2+ miR491 inhibitor
Relative invasion
MGC803
AGS
pc-NC
pc-CASC19
pc-CASC19+ miR491 mimics
HGC27
pc-NC
pc-CASC19
pc-CASC19+ miR491 mimics
Relative invasion
D
si-NC
si-CASC19-2
si-CASC19-2+ miR491 inhibitor
MGC803 PE-A
FITC-A
Q1-UL(0.61%)
Q1-UR(1.80%)
Q1-LL(93.75%)
Q1-LR(3.84%)
Q1-UL(7.01%)
Q1-UR(11.15%)
Q1-LL(78.84%)
Q1-LR(3.00%)
Q1-UL(1.87%)
Q1-UR(3.98%)
Q1-LL(90.02%)
Q1-LR(4.13%)
MGC803
Percentage of apoptosis (%)
si-NC
si-CASC19-2
si-CASC19-2+ miR491 inhibitor
AGS PE-A
Q1-UL(0.96%)
Q1-UR(1.24%)
Q1-LL(95.17%)
Q1-LR(2.63%)
Q1-UL(1.80%)
Q1-UR(7.83%)
Q1-LL(88.10%)
Q1-LR(2.28%)
Q1-UL(2.21%)
Q1-UR(5.89%)
Q1-LL(87.76%)
Q1-LR(4.14%)
AGS
E
MGC803
si-NC
si-CASC 19-2
si-CASC19-2+ miR491 inhibitor
AGS
si-NC
si-CASC 19-2
si-CASC19-2+ miR491 inhibitor
Survivin
Bcl-2
Caspase 3
Caspase 9
GAPDH
Relative expression of survivion
MGC803
AGS
si-NC
si-CASC19-2
si-CASC19-2+ miR491 inhibitor

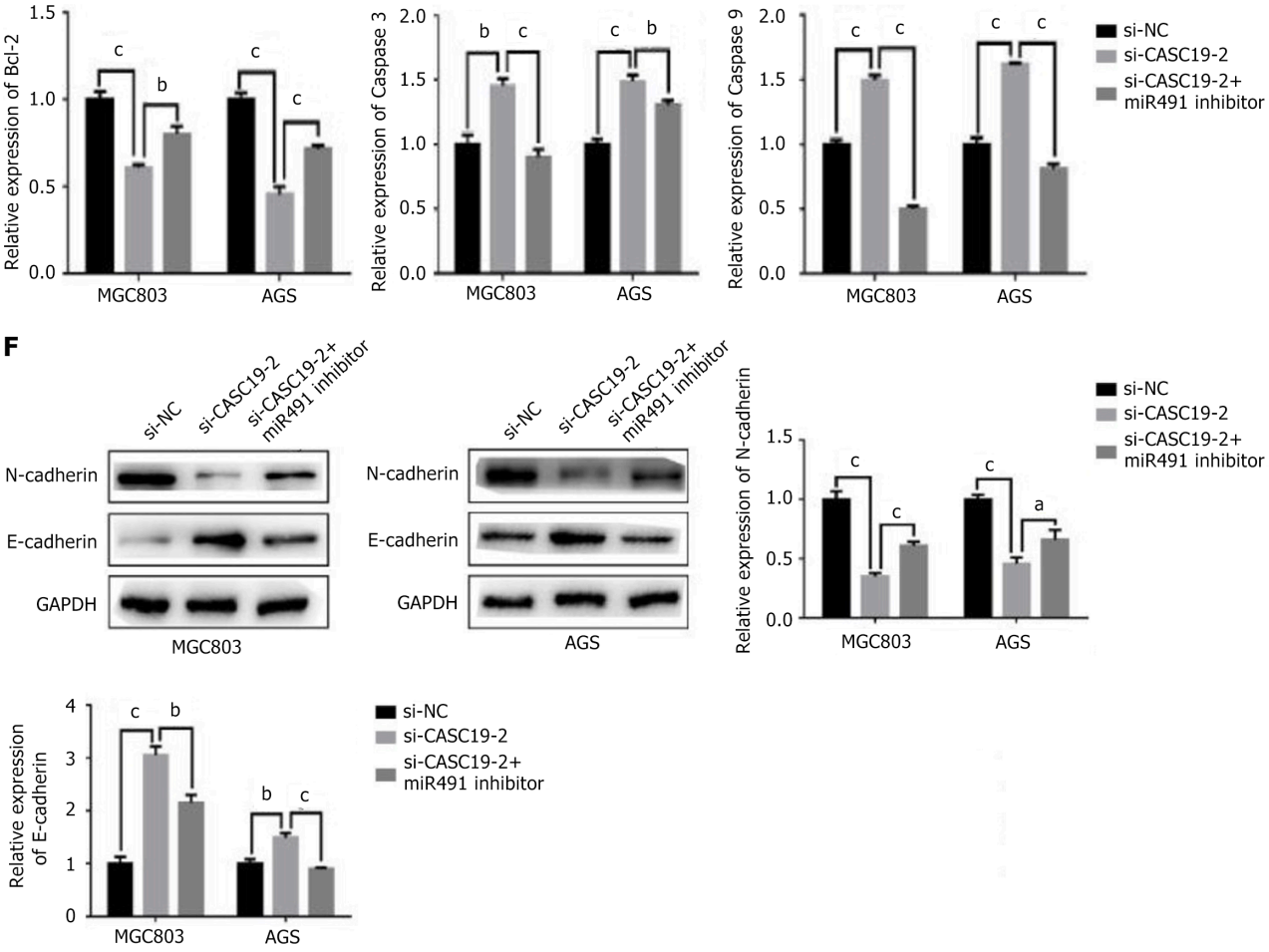
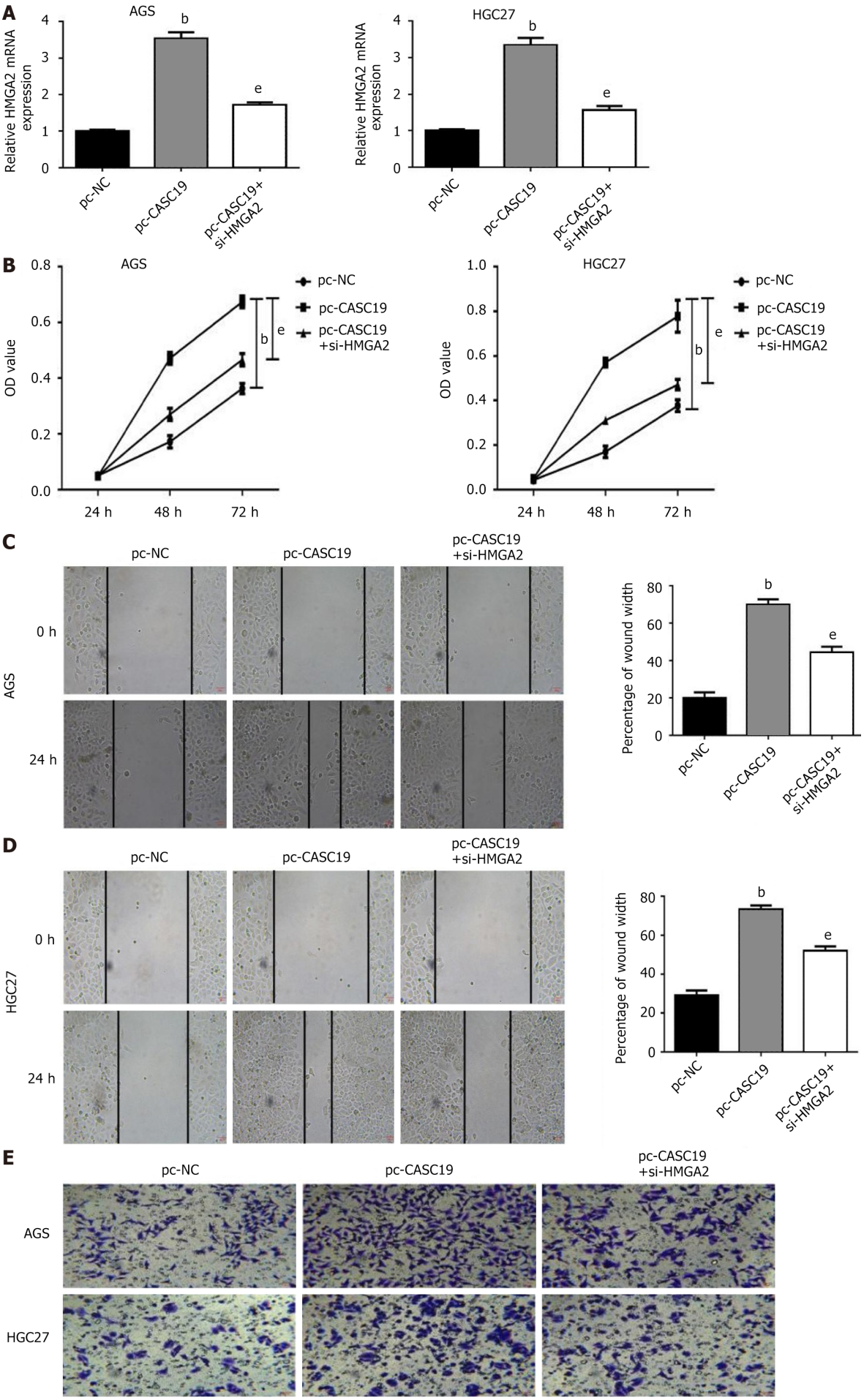


Figure 6 The ability of miR-491-5p to rescue CASC19 in gastric cancer cells. A: The cell proliferation ability was detected by cell counting kit-8; B: The migration ability of cells was detected by wound healing assay; C: The invasion ability of cells was detected by transwell; D: Flow cytometry was used to detect cell apoptosis; E: The level of apoptosis-related proteins were detected by western blot experiment; F: The level of epithelial-mesenchymal transition-related proteins was detected by western blot experiment. $^{a}P < 0.05$, $^{b}P < 0.01$, $^{c}P < 0.001$, $^{d}P < 0.05$, $^{e}P < 0.01$ was compared with the control group respectively, $P < 0.05$ was considered statistically significant.

the expression of CASC19 was down-regulated, the expression of HMGA2, N-cadherin and Ki-67 was significantly down-regulated, while the expression of E-cadherin was significantly up-regulated (Figure 8E). RT-PCR was performed on the tumor to examine the expression levels of CASC19, miR-491-5p, and HMGA2, and it was found that the expression of CASC19 was down-regulated, the expression of HMGA2 was significantly down-regulated, and the expression of miR-491-5p was significantly up-regulated (Figure 8F).

DISCUSSION

The treatment of GC has been greatly improved in recent years, but its clinical treatment effect is not very satisfactory. Therefore, it is particularly important to explore the mechanism of progression of GC and to find new markers. The abnormal expression of LncRNA has been reported and studied in various tumors[30,31], increasing research on the function and regulation of lncRNA will help discover new diagnostic methods and therapeutic targets. Many studies have confirmed that CASC19 is involved in the progression of cancer, including colorectal cancer and prostate cancer[32, 33]. In recent years, it has been found that CASC19 can regulate the level of miRNAs to regulate gene changes and thus affect tumor progression, such as CASC19 adsorbs *miR-140-5p* to regulate the expression of CEMIP in colorectal cancer [34], but the mechanism of CASC19 binding miRNA regulatory genes in GC has not been reported. Therefore, it is worthy to explore the effect of CASC19 on GC. The expression of CASC19 in GC tissues and GC cells is higher than that in normal tissues and cells, respectively. The expression level of CASC19 is related to the patient's T stage, N stage and tumor size, therefor CASC19 is correlated with the pathological characteristics of GC. Besides, CASC19 is a risk factor that affects the prognosis of GC patients. Therefore, CASC19 can be used as an important indicator for judging prognosis in clinical practice. CASC19 can inhibit the proliferation and invasion of GC cells by Knockdown of the expression of CASC19, besides *si-CASC19-2* can inhibit EMT transition and promote the apoptosis of GC cells. Based on the above findings, it is proposed that CASC19 may be a potential marker for evaluating the malignancy of GC. *MiR-491-5p* is also novel miRNA that was engaged in CASC19-mediated GC progression, which can induce apoptosis and inhibit the prolif-


A
AGS
HGC27
Relative HMGA2 mRNA expression
pc-NC
pc-CASC19
pc-CASC19+ si-HMGA2
B
OD value
24 h
48 h
72 h
C
D
0 h
24 h
Percentage of wound width
E

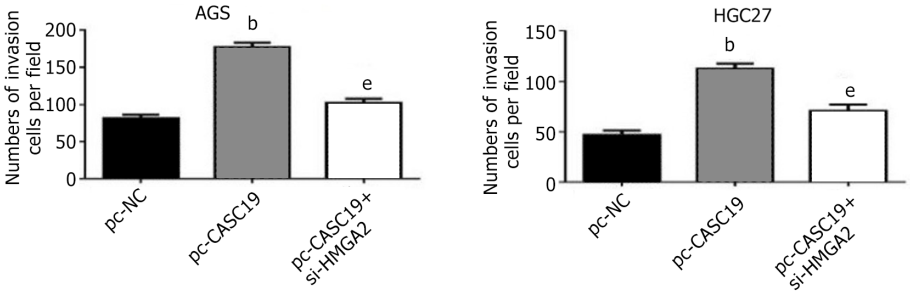
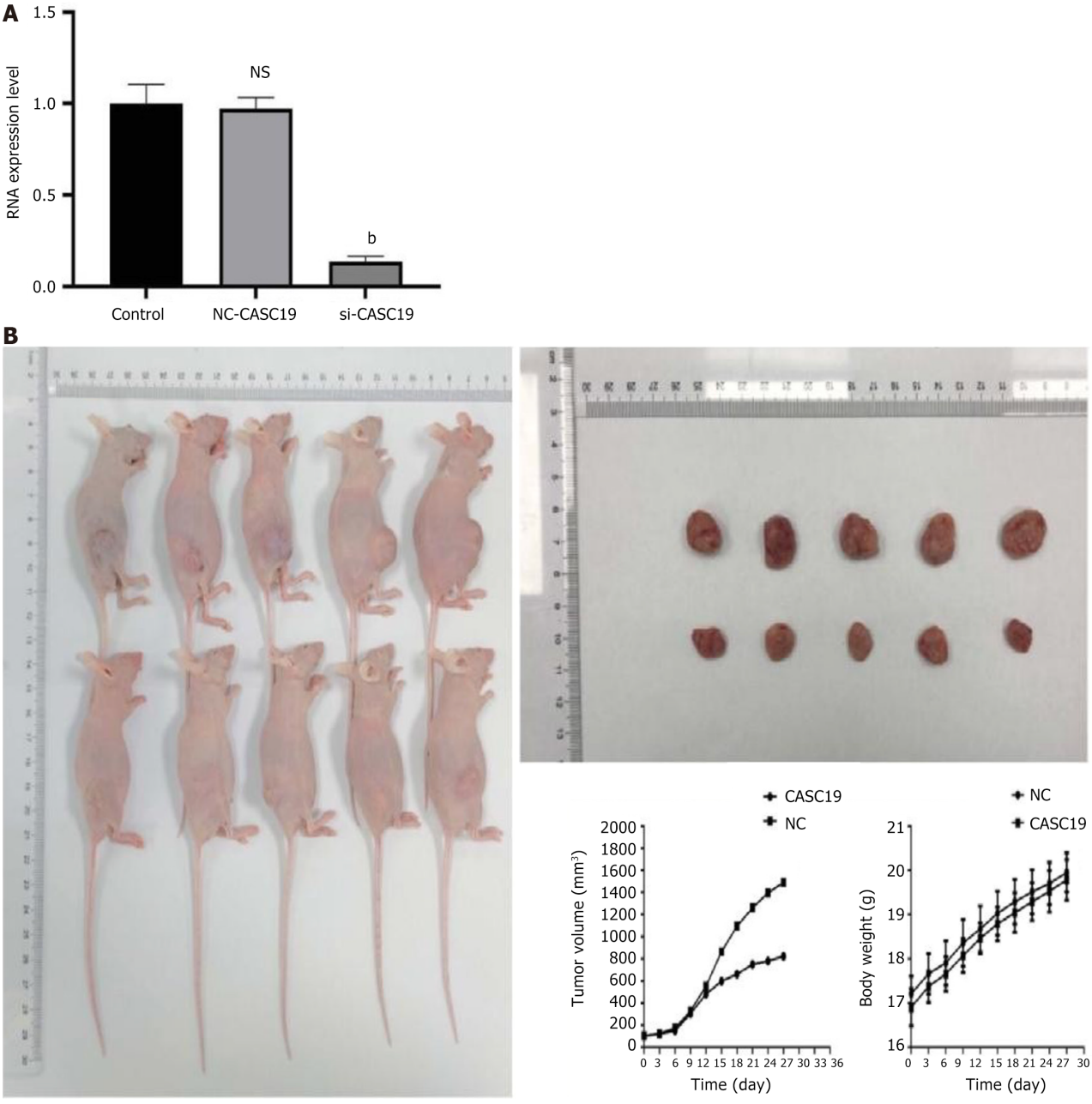


Figure 7 HMGA2 can rescue the biological behavior of CASC19 in gastric cancer cells. A: Reverse transcription polymerase chain reaction revealed the expression of HMGA2; B: Cell proliferation was detected by cell counting kit-8; C and D: The migration ability of AGS and HGC27 cells was detected by wounding assay; E: The invasive ability of the cells was detected by transwell. $^{b}P < 0.01$, $^{e}P < 0.01$ was compared with the control group respectively. $P < 0.05$ was considered statistically significant.



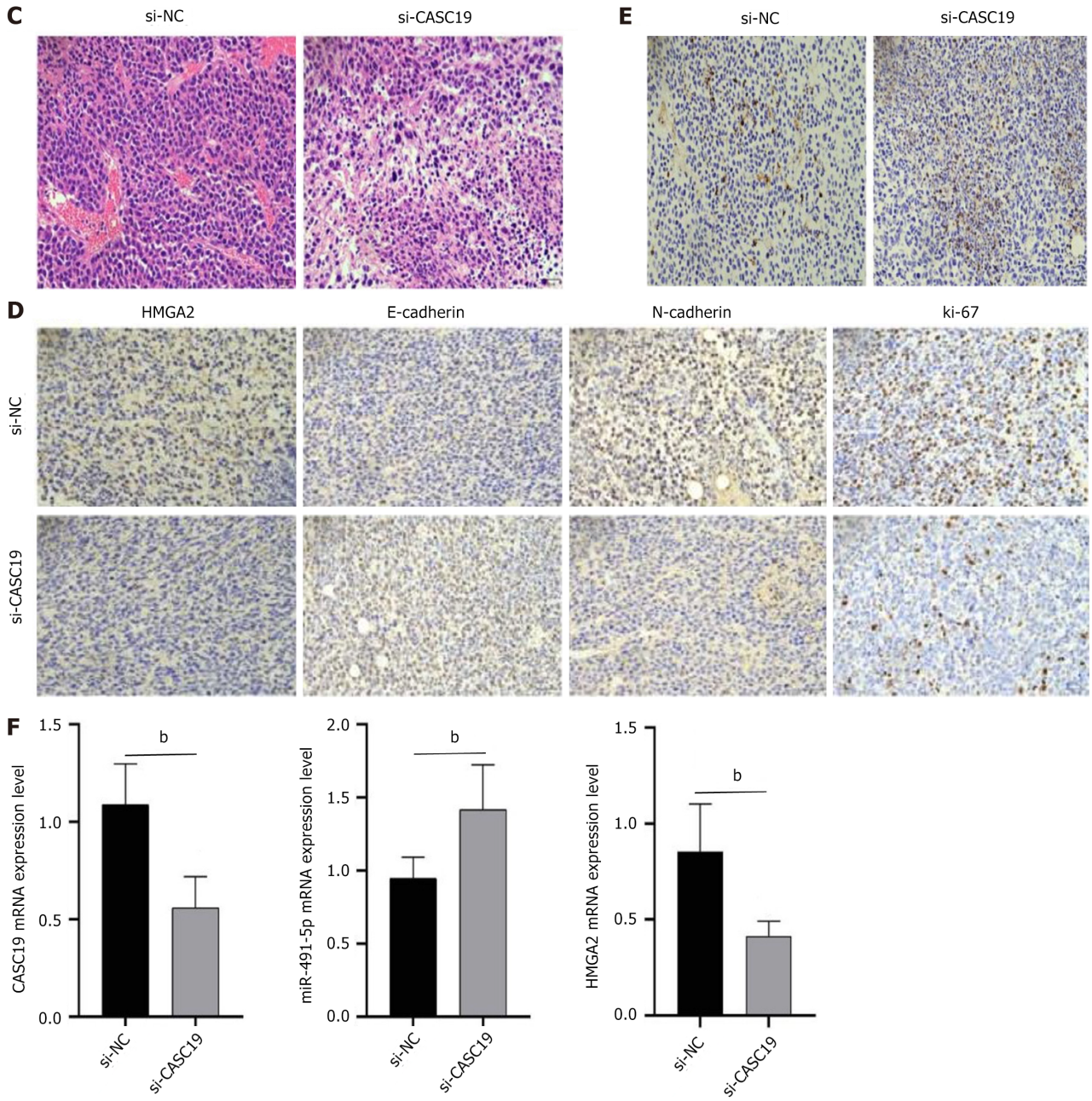


Figure 8 The oncogenic mechanism of CASC19 to gastric cancer *in vivo*. A: The efficiency of lentivirus silencing CASC19 was verified by reverse transcription polymerase chain reaction (RT-PCR); B: The tumor growth rate of the CASC19 silencing lentivirus infection group was slower, and the tumor weight was significantly smaller than that of the negative control group; C: The hematoxylin and eosin staining of the tumor; D: Tumors were stained with TUNEL; E: The tumor was subjected to immunohistochemical staining; F: RT-PCR was performed on the tumor to examine the expression levels of CASC19, miR-491-5p, and HMGA2. $^{b}P < 0.05$ was compared with the control group and $P < 0.05$ was considered statistically significant.

eration of pancreatic, ovarian, breast and colorectal cancer cells, as well as the migration and migration of gliomas and breast cancer[35,36]. However, The role of *miR-491-5p* in GC, especially in the cell cycle, apoptosis and EMT of GC, is still unclear[37]. Our study revealed that increasing the expression of *miR491-5p* can inhibit the proliferation, EMT transition and promote the apoptosis of GC cells. HMGA2 (also known as HMGI-C) is a protein that binds to DNA as a structural transcription regulator, and can bind to AT-rich conserved sequences[38]. HMGA2 is very important in the process of embryonic morphogenesis[39]. Studies have shown that HMGA2 can not only play a regulatory role in benign fibrous histiocytoma, nodular fasciitis, aggressive angiomyxoma and acute myeloid leukemia[40,41]. HMGA2 can also regulate the expression of oncogenes and tumor suppressor factors[42]. HMGA2 can influence a variety of biological processes in cancer, including the cell cycle process, DNA damage repair process, apoptosis, senescence, epithelial-mesenchymal transition and telomere restoration, the overexpression of HMGA2 is a feature of malignancy[43]. Our study got the same results, the expression of HMGA2 in GC tissues is higher than that in adjacent tissues. At the same time, the expression level of HMGA2 is related to the patient's N stage and T stage. Reducing the level of HMGA2 can inhibit the proliferation and promote cell apoptosis, indicating that HMGA2 plays an important biological function in GC. Recently, more and more evidence has shown that tumors Immune infiltration is a promising target for tumor treatment[44,45], immune

infiltration cells can suppress macrophages to Kill cancer cells in the tumor microenvironment[46]. By analyzing the expression of HMGA2 and the level of immune cells, it is found that B cells memory, T cells CD4 memory resting, T cells CD4 memory activated, T cells follicular helper, T cells regulatory (Tregs), Macrophages M1, Mast cells resting are related to HMGA2 ($P < 0.05$), so the level of HMGA2 may be related to immune infiltration. Besides, the multi-GSEA results showed that HMGA2 is corrected with immune-pathways such as intestinal immune network for IGA production, natural killer cell mediated cytotoxicity, T cell receptor signal pathway. HMGs were tightly associated with immune infiltration and tumor immune escape in HMG-mediated oncogenesis in GC[47], then we speculated HMGA2 facilitated immune infiltration and Tumor microenvironment to promote the progression of GC. We use biological information to predict that CASC19 may regulate the level of HMGA2 through miR491 to affect the progression of GC. Then this hypothesis was verified *in vivo* and *in vitro* experiments. First, the targeting relationship between CASC19 and *microR491-5p*, *microR491-5p* and HMGA2 was proved by the luciferase report and PCR, indicating that the expression level of HMGA2 is regulated by CASC19 and *micro491-5p*, besides, enhancing the expression of *miR491-5p* can rescue the effect of pcDNA-CASC19 on HMGA2, inhibiting the expression of *miR491-5p* can rescue the inhibitory effect of *si-CASC19-2* on HMGA2. The luciferase results showed that overexpression of CASC19 could block the inhibitory effect of *miR-491-5p* on HMGA2 3'UTR relative luciferase expression, suggesting that CASC19 could attenuate the effect of *miR-491-5p* on HMGA2 by competitively binding to *miR-491-5p*, thereby confirming the ceRNA function of CASC19. This shows that CASC19 can down-regulate the expression of *miR491-5p* to increase the level of HMGA2. HMGA2 plays an important role in GC, and has related effects on the regulation of GC cell proliferation, invasion, migration, apoptosis, EMT and other biological behaviors, HMGA2 overexpression promotes the stemness acquisition of stem cells from GC cells[48]. Besides, previous study showed that *miR491-5p* can affect the proliferation and invasion of GC through HMGA2, in order to further verify the regulation of CASC19 on *miR-491-5p*/HMGA2, the rescue function of *miR-491-5p* on the biological behavior of CASC19 in GC cells was verified by CCK8, transwell, apoptosis and other experiments. *Micro491-5p* inhibitor can rescue the inhibition of *si-CASC19-2* on the proliferation, invasion and migration of GC cells. At the same time, *miR-491-5p* mimics can rescue the ability of pc-CASC19 to promote the proliferation, invasion and migration of GC cells. Simultaneously si-HMGA2 can rescue the ability of pc-CASC19 to promote the proliferation, invasion and migration of GC cells. Therefore, CASC19 can affect the proliferation, invasion and migration of GC cells through sponge *miR-491-5p* and then regulate HMGA2. We can use *miR-491-5p* inhibitor to rescue the effects of *si-CASC19-2* on apoptosis and EMT conversion of GC cell. *Si-CASC19-2* can increase the expression of caspase3, caspase9 and E-cadherin, while decrease the expression the expression of survivin, BCL-2 and N-cadherin, adding the *miR491-5p* inhibitor can reverse this change, so CASC19 can affect HMGA2 through *miR491-5p* to regulate the apoptosis and EMT transition of GC cells. This signal axis may provide new insights into the mechanism of GC progression and target therapy. This study has some weakness. The *miR491-5p*/HMGA2 axis may only be one of the mechanisms of CASC19 regulating GC, and other potential downstream factors should be explored in the future. In the cell function experiment, the expression level of HMGA2 and *miR-491-5p* in cells was changed by transient transfection of siRNA or miRNA mimics, but this was not achieved by stably transfected.

CONCLUSION

LncRNA CASC19, *miR491-5p* and HMGA2 play important role in the development of GC, *LncRNA CASC19* regulates the expression of HMGA2 through *miR491* to affect the proliferation, invasion, migration, apoptosis and EMT transition of GC. Besides, the function of HMGA2 related to immune microenvironment which may be regulated by the *LncRNA CASC19*/*miR-491-5p* axis, this needed further study.

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FOOTNOTES

Author contributions: Zhang LX write the main work; Luo PQ helped finish the experiments and write the manuscript; Xu AM designed this study; Wei ZJ and Guo T revised this manuscript; Zhang LX and Luo PQ contribute this work equally as co-first author; Xu AM and Guo T contribute this work equally as co-corresponding authors; all authors read and approved the final manuscript.

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